Foreword

THE publication of this brochure represents an attempt by some of Dr. Gilbert John Fowler's past and present students to mark their appreciation of his qualities of head and heart, on the eve of his retirement from the chair of Bio-Chemistry at the Indian Institute of Science. One of them has enjoyed association with him for more than 35 years and although the others can claim this privilege for a much shorter period only, all unite in testifying to the unfailing courtesy and help they have received from him; they believe that this appreciation could best be expressed by assembling some selected results of investigations in which he has been interested.

This collection has been compiled and dedicated to him in the hope that he will recognise in it an expression of the desire he has created for exact knowledge and for the application of that knowledge to useful ends. May he continue to assist with sympathetic advice and may he live long to see the full fruition of the seeds he has planted.

CONTENTS

I

STUDIES IN THE PHYSIOLOGY OF THE ACETONE ORGANISM

BY

V. Subramanyam, B.A. (JUNR.)

DURING the war, large quantities of acetone were manufactured in India and elsewhere by fermenting cereals with a bacterium which was isolated by Dr. Weizmann in England. In our present study, we have attempted to define the Physiological characteristics of the Weizmann bacillus with a view to comparing it with the allied organisms, to determine its origin, function and condition of maximum activity and finally, to sketch the interesting cycle of its existence in nature. For our experiments the culture from one of the spore tubes brought from England was used which though eight years old was found to develop very well on jawari mash.

The organism is a short thin rod, which is motile. Its spores are highly thermo-resistant, being capable of standing 100° C. for more than 2 minutes and 60—65° for five to six hours. It does not grow on any of the common media nor can it be easily plated out. The only medium on which it thrives well is the cereal mash, primarily that of maize, jawari and paddy. The mash is best prepared by first cooking the powder with some water for one hour at four atomspheres, then diluting to the required volume and finally sterilising at 10 lbs. pressure.

A study of the food factors required by the organism shows that it requires a carbohydrate, primarily strach; that it needs a vegetable protein, particularly that which is not soluble in water, the soluble proteins being invariably left unattacked; and that stimulants, e.g., amino compounds, do not very much enhance its activity (calcium

carbonate suppresses its growth). The initial acidity of the medium is very important, the best results being obtained with acidity 0·1 (·1 c.c of N. alkali for every 100 c.c of the mash).

The organism is a facultative anaerobe. It can be made to grow and multiply like the yeasts, with aeration without producing any fermentation. Some critical experiments show that during the fermentation, the enzyme concentration begins at a certain point and gradually extends to the other parts of the fermenting medium.

Some experiments were conducted with a view to determining the nature of the amylase present in the organism. Glucose is invariably the sugar formed from the degradation of starch, maltose, and the dextrins. The dextrins which are the general intermediate products during starch hydrolysis, cannot, at any stage, be observed. Some experiments are afoot to determine if the organism assimilates the amylose or the amylo-pectin of the cereal starch and to compare the fermentability of maltose with that of isomaltose. (Amylo-pectin is got as the residue after the dialysis of the starch. Isomaltose is obtained from it by first attacking it with diastase of malt. The sugars thus obtained are attacked with S. Cerevisea. The residue is concentrated and precipitated with ninetysix per cent. alcohol. The precipitate thus obtained is isomaltose). Attempts to detect the presence of a peroxidase and a reductase in the organism have not met with success.

As a result of our study of the post-fermentation changes we find that the abnormal increase in acidity to 3·9 and the unaccountable disappearance of acetone from the flask, on keeping, are linked together and are mostly due to the bacterial action.

Attempts at fermenting the mashes in symbiosis with yeast and fungi have not yielded much encouragement. The yield of acetone is low, while the yield of alcohol is not particularly high.

Some experiments were conducted to ascertain why the fermentation of Mahua flowers by the organism cannot be made a practical success. Our results show that the extract of Mahua is unfermentable because of the presence of tannins which the organism cannot assimilate, and the absence of the insoluble protein which the organism so much requires. The essential oil does not inhibit the growth of fermentation. The residual mash after the extraction of the soluble matters is somewhat fermentable, the yield of acetone being 3·4 per cent. It may also be fermented as mixed with starch. The Mahua waste left behind after yeast frementation is unfermentable. Symbiotic fermentation with yeast gives a 6—7 per cent. yield of acohol and 1·5—2 per cent. yield of acetone (on the total weight of solid matter taken).

The residue from fermentation of the cereals was cooked and fermented with some xylose ferments. The yields do not exceed 1·7—1·9 per cent. of alcohol or acetic acid.

Two organisms (acetone-producing) were isolated (following Weizmann's method of selective sterilisation) from paddy field soil and potatoes. Detailed study of their morphology and physiology remains to be done and further work is in progress.

II

THE RETTING OF COIR.

BY

F. Marsden, Ph.D., M.Sc.

COIR is a fibre employed in brush, rope and mat making and is extracted from the mesocarp or husk of the cocoanut. In this the fibres lie longitudinally from base to tip and vary in length and thickness according to their position in the husk; they are embedded in and held together by a pith or cork-like cellular structure which is white, soft and elastic in the freshly plucked nut but upon drying out becomes brownish-red and hard.

In most districts where cocoanuts are plentiful the fibres are separated from this pith (for the purposes of making rough cordage) by beating and crushing, but the product obtained in this way is always harsh and deeply coloured. In the Laccadive Islands and on the West Coast of India where the water conditions are suitable, a much finer, paler and softer fibre is obtained by soaking the husk in water for some months whereby the pith is softened and to a large extent destroyed; the fibres are then separated by beating and obtained in a condition suitable for spinning into string and rope.

In Malabar and Travancore this separation and spinning and weaving of coir is an important industry about which little was known or published when the Department of Industries, Madras, became responsible for the supply to the Munitions Board during the War (1914—18). In 1920 it was decided to investigate the soaking process with a view to its acceleration if possible, for it appeared to be bacterial in character and capable of extension. A tour of the chief producing centres was made and samples of husk in soak, and water from various centres, were collected for examination at Bangalore.

The cocoanuts are usually collected when about 10 months old, or just short of full ripeness, but this depends upon the relative conditions of the oil and fibre markets, the general opinion among the workers being that unripe nuts give better fibre than ripe ones. After collection the husks are split by forcing them upon the point of a firmly fixed stake so that this penetrates between the kernel and the husk and with a sharp twist, the husk is loosened; three "cuts" are sufficient to separate the kernel and husk completely.

After splitting, the husk pieces are placed in pits dug in the sand of the river banks or backwaters, or in submerged fields in which a change of water is caused by the rise and fall of the tide or they are dumped into staked areas in the backwaters themselves or sunk in the tideway enclosed in large coir nets. It is well recognised that light-coloured coir is not obtainable from husk which has been exposed, after splitting, to the influence of the weather, and that the fibre from unripe nuts tends to be deeper in colour than that from ripe ones. The general impression is that the nature of the water is an important factor in the retting process and that the best results are obtained where, from the local conditions, the soaking is conducted in water which is partly salt and partly fresh, and that fresh water alone is not suitable as the fibre shows a tendency to dullness in colour. Consequently husk is not set to soak in the rainy season when the rivers are in flood and the water in the backwaters fairly fresh.

The course of the retting is somewhat as follows:— The husks are submerged with as little delay as possible in the soaking pit and during the first few days the water is coloured brown by the matter which is dissolved out. As this colour disappears a white growth commences and in about a fortnight has become pronounced and a smell of sulphuretted hydrogen is noticed. Gas bubbles begin to rise and the smell and gas formation increase during 3—4

months after which they gradually die down until after six months they almost disappear. Upon lifting at this stage the husks are found to be quite soft except for the portions lying next to the outer skin (or epicarp) and at the tip, and for the proper softening of these the husks are left submerged for a few months longer. The fibres can now be separated from the residual pith by squeezing in the hand and are ready for beating out; the husks are lifted, rinsed in clean water to remove the dark slime which covers them and are then drained and beaten with a wooden mallet or a flat stone. The fibres and pith are thus thoroughly loosened and after willowing (for dusting out the dry pith) the fibres are roughly carded and sorted into the coarse brush and bristle fibre and the finer portion goes forward for spinning.

The examination of the West Coast water samples demonstrated the presence of numerous types of organisms, as was only to be expected from the conditions of the areas from which they were taken, and although upon plating out some types of colony appeared common to all, it might have been difficult to decide which were responsible for the retting effect. The investigation was much simplified, however, by the observation that a piece of husk which had been obtained and set to soak in tap water developed a smell, and growth similar to that noticed in the retting pits. This indicated that there was no special virtue inherent in the waters of the West Coast, that salt water was not essential for the purposes of retting and that the organisms which were present in the husk itself might be capable of destroying the binding matter in the pith.

To test the first two points a supply of nuts was obtained locally, split and the husk set to soak in a large enamelled iron vessel in which the water could be changed at intervals. The phenomena were exactly as above described,—first a reddish brown coloration of the water, followed by a white growth which later developed into a

jelly-like mass and an evolution of gas smelling like sulphuretted hydrogen. Under the microscope the jelly was seen to be nothing but an agglomeration of bacterial nature, the bacteria being of the nature of long rods lying in sheaths, the threads intertwining and forming a soft mass which at first appeared like the soft immature flesh inside the kernel of the nut. As time went on the growth lost its white appearance and became dark grey and slimy, but the softening of the husk progressed until the fibres could be easily freed from the pith.

It was clear therefore that the retting effect could be studied upon Bangalore nuts and Bangalore water and that the effects were similar to those supposed to be obtainable only on the West Coast. There remained the question of the source of the retting organism and to solve this, experiments were set on under sterile conditions to determine whether the organisms in the husk itself were not capable of producing the effect.

It was also desirable to obtain informations regarding the constituents of the husk and with this in view husk-cuttings were extracted and the extracts examined. The most prominent constituent was a tannin which was difficult to extract completely, and the solution of which darkened rapidly and on evaporation or treatment with acid deposited an insoluble red precipitate reminiscent of phlobaphene. Ether extracted a small quantity of oil and wax, and from the ether-extracted material alcohol removed tannin and a sugar which gave an osazone, m.p. 205°, with the characteristic appearance of that of glucose. Extraction with water at 100° C. completed the removal of soluble tannins, but nothing else was found in the extract except traces of a calcium salt. The husk-cuttings were still hard and compact, so the extraction was continued by heating with water in an autoclave under 40—50 lbs. pressure. The operation was repeated with fresh quantities of water until the husk was quite soft and the extract obtained was only faintly coloured; the mixed extracts

were then examined. They were distinctly acid to litmus and upon evaporation on the water bath a deposition of matter of a dark colour commenced when approaching dryness. Addition of alcohol to a portion of the solution gave a precipitate reminiscent of gum and showing the pentose reaction on distilling with hydrochloric acid.

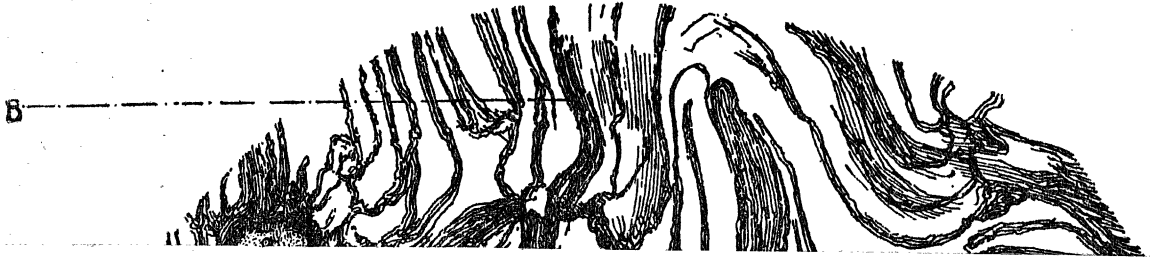
The bulk of the solution was hydrolysed with sulphuric acid, treated with barium carbonate, filtered and evaporated. Upon taking up with alcohol, a residue remained which was soluble in water and behaved like the barium salt of a gum-acid. The alcoholic solution contained a sugar which gave an osazone, m.p. 160°, and showing the rotation (α) D $^{25°} = +17{\cdot}4°$ and characteristic crystals (upon treatment with bromine and cadmium carbonate) of xylose.

The husk autoclaved with water, was again heated under pressure with a 2 per cent. solution of sulphuric acid and from the extract a gum-acid was obtained and a sugar recognised as glucose by the form and melting point (205°) of its osazone.

The husk pieces were disintegrated by the treatment, the fibres being quite free, and it would appear therefore that the binding matter contained in the pith in which the fibres of the husk are embedded is of a gummy nature, and is removable either by severe chemical treatment or by bacterial action.

The presence of bacteria in the husk of Bangalore cocoanuts was proved by selecting a nut with undamaged epicarp direct from the tree, washing and sterilising the surface by flaming and with all sterile precautions taking cuttings and placing them in a closed sterilised vessel through which sterile water could be passed. The results were identical with those observed in the larger, laboratory experiment with unsterilised husk and water.

Cuttings from a fresh nut were also taken with all sterile precautions, embedded in nutrient agar in Petri


B

dishes and incubated at 36° C. Growth of mass colonie proceeded from the edges of the cuttings, the colonie being raised, moist, with lobed edges, but a special care i sterilising the nut was essential as the surface seem (notably when dried out a little) to be heavily infected b moulds. Inoculations of the characteristic colonies wer made into glucose-nutrient-agar and glucose-peptone an from these it was seen that the organism was a rod bac terium, with a tendency to form chains reminiscent c *Sphoerotilus* but its identity with any species of this grou has not yet been proved. The colonies on plates prepare with inoculated nutrient agar had a granular structure were round, raised and moist when on the surface but wer centicular or angular in shape when growing below th surface of the medium.

For purposes of comparison, cocoanuts of differen varieties with undamaged husks were obtained from widel separated areas in Travancore and the North-East of th Madras Presidency. They were sterilised on the surfac by washing with sterile water, then with formalin an finally with the alcohol remaining on the husk being ignited and burnt off. Cuttings were then taken from the lower layers of the husk and placed, some on t nutrient agar in Petri Dishes, some in sterile water i plugged test tubes, as reserve.

The growths on nutrient agar appeared to be affected somewhat by the tannin content of the husk, but after lying in sterile water for 24 hours and then transferring to nutrient agar and incubating, the growth described with Bangalore husk was seen to be common to all, no matter what the district from which it was obtained. It would appear therefore that the cocoanut carries a form of bacteria peculiar to it, which is capable of attacking and destroying the binding matter of the mesocarp and exerts its activity when the husk is kept moist, thus assisting the exit of the sproutlet through the husk in germination ; for as the nut ripens the cork-like cellular matter at the

suspension end of the nut disappears and the fibres become separated.

The investigation has demonstrated that:—

1. The cocoanut carries bacteria which are capable of destroying the binding matter of the husk, so that the fibres are easily separable.

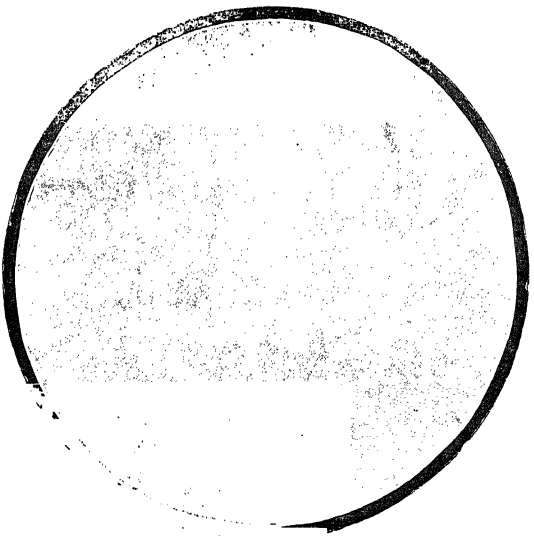
2. Whatever the locality in which the palm grows in the South of India, there is no difference in the nature of the bacterial content of the nut.

3. Husk from ripe nuts is more rapidly broken down than that from unripe ones.

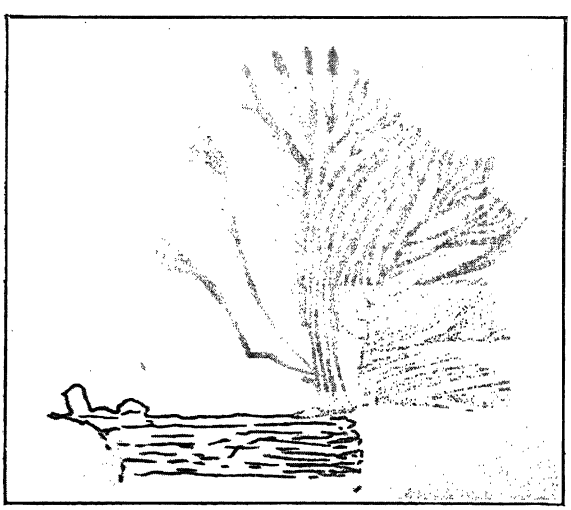
4. The matter binding the fibres in the husk is of an insoluble, gumlike character and is associated with the pith or cork-like cellular structure in which the fibres lie.

5. The variations in colour of the coir produced in the ordinary retting process are due to the soluble constituents. Easily oxidisable tannins, readily converted into a red insoluble phlobaphene-like substance, cause the reddening of the coir and explain the necessity of bringing the husk into soak immediately after splitting. These tannins also explain the production of dull-coloured coir when floods occur just after the husk is set to soak; apart from the larger quantity of air which may be dissolved in this fresh water compared with that in the brackish backwaters, iron-compounds are carried in the flood water from the laterite soils and, reacting with the tannin, this iron gives the " blue water" which results in " grey" coir.

6. The retardation of the rate of retting after floods is due to the washing away of the bulk of the bacterial growth and the lowering of the temperature in the mass of husk by the cold water. This temperature may normally rise to 40—45° C. and the gas evolution is rapid, but after flood it takes some time for suitable conditions to re-establish themselves.



Retting Bacteria



Growth on Nutrient Agar.

For the efficient retting of coir husk, the conditions would seem to be therefore the selection of ripe nuts, the placing of the husk to soak as soon as possible after splitting, regulation of the water flow so that there is no washing away of the established bacterial flora but sufficient change to remove soluble waste and gaseous products, and maintenance of the temperature (by protecting the soaking areas from the effects of flood-water), incidentally thus preserving the brightness of colour of the coir.

III

BIOCHEMICAL CONTROL OF THE VINEGAR INDUSTRY

BY

V. Subramanyam, B.A. (SENR.)

IN an extremely interesting article on "Micro-Organisms and some of their Industrial uses" (J. Roy. Soc. Arts, Vol. 69, P. 604) A. Chaston Chapman declares that :—

"Notwithstanding the great advances made in many directions in industrial Zymo-Technology as a result of improved methods of bacteriological technique and in consequence of the greater amount of attention devoted, during recent years, to this important subject, the position of the vinegar industry has almost remained stationary. Apart from the crude apparatus they employ, vinegar-makers rarely, if ever, know anything of the precise character of the all important organism (or organisms) they are using, nor, except in a very rough way, do they know the most suitable conditions for obtaining—through its agency—the maximum yield of acid". Again Lafar, an authority on technical mycology, developing the theme on almost similar lines, says: "This highly necessitous industry has, more perhaps than any other, to struggle against a variety of difficulties; the actual losses of alcohol are enormous, and no one is able to offer any reliabe explanation of their cause. The introduction and intelligent use of pure culture ferments would be a great boon."

The above two quotations will suffice to show, how much remains to be done to put this ancient and interesting industry on a really satisfactory bio-chemical basis and hence are sufficient plea to indicate in the following few pages recent lines of developement of

the subject in the laboratories of the Indian Institute of Science. The author does not lay any claim to have perfected a process for the manufacture of vinegar, but enough has been done on the simple bacterial conversion of alcohol into acetic acid, which in turn has opened up increasing possibilities of application.

Several attempts have been made in these laboratories before, on the same lines; but they have resulted in failures owing to the fact that the organism or organisms employed were not systematically studied with regard to their nutrition, growth, and functional activity. It is this significant fact, which has not received much attention, not only in the hands of vinegar-makers but also in those of bacteriological chemists, that has shrouded the whole subject in mystery and made the manufacturers "trust to luck."

The conversion of alcohol into acetic acid is one of the simplest bacterial oxidations and a large number of organisms have been found to affect it. How far all of these could be looked upon as different species in a biological sense it is difficult to decide; but apart from morphological considerations the biochemist looks upon the organism as an effective chemical agent and his efforts are directed towards obtaining the greatest concentration of enzymic activity. The organism that has been employed in these laboratories is one of a numerous species *B. Aceti*, Hansen, which was obtained from a medium composed of suitable proportions of alcohol, acetic acid (vinegar) and mineral phosphates of potassium, ammonium and magnesium. On exposure to air in an incubator at 35° C., they grow very easily and purification for experimental purposes has been accomplished by combined plating and dilution methods. After purification the cultures were inoculated into larger and larger volumes of medium and in this way considerable quantities of the organism were built up as a fine grey deposit at the bottom of the culture flask. By

slowly increasing the concentration of acetic acid in the medium, the acid-sensitive bacteria were eliminated and the acid resistant ones, which are the most important for acetification purposes, were favoured.

In the course of preparing the cultures used in the work, a number of interesting observations were made as to the most suitable nutritive material for the bacteria. It should be understood that the requirements vary as the object in view is to produce growth in bulk or to encourage functional (acetifying) activity. The former condition obtains when preparing large masses prior to the production of acetic acid, the latter as soon as acetification begins. Mineral phosphates such as those of magnesium and ammonium, in presence of suitable quantities of glucose, have been found to increase the growth activity at the expense of acetification. Accordingly these phosphates have been omitted in conducting acetification experiments and the proportions of ammonium phosphate and glucose have been reduced to a minimum to prevent starvation.

Having obtained pure cultures of the right organism in quantity experiments were carried out, with dilute alcohol of specific strength 4 per cent. in the absence of artificial aeration. Good results have been obtained and the influence of catalysts like ethyl acetate and manganese sulphate, the former extending the acetification to a greater maximum acidity and the latter quickening the period of acetification has been very beneficial. Carrying on the experiments further with increased volumes of inoculant and acetifying liquid the necessity for artificial aeration became increasingly marked, due to lack of insufficient oxygen. It was also evident from observations of sundry cultures kept without regard to aeration that if large quantities of inoculant were to be used it would be necessary to introduce air artificially to prevent the

putrefaction of the bacterial masses. The conditions of maximum efficiency to be aimed at, would therefore be the employment of the highest quantity of inoculant which would be kept by adequate food supply, and sufficient oxidation to prevent putrefaction, or even the production of intermediate products, but insufficient to encourage super-oxidation to carbon dioxide. Experiments were carried out on these lines using a bottle with an outside temperature 32° C. and with air supply from a filter pump. Further alcohol was added as the initial alcohol became oxidised and working this way under sterile conditions an acidity of 10 to 12 per cent. of acetic acid was obtained.

The experiments were then translated to a larger scale using an inverted bell-jar and with air from a gas-holder. The exact translation has not been however possible but working this way an eventual acidity of 9 to 10 per cent. was obtained.

Attempts were now made to approach actual conditions of working on a large scale, in order to anticipate some of the difficulties involved therein chiefly by way of infection from outside. For these experiments a ten-gallon tub was used. To avoid blowing much air and at the same time to ensure thorough mixing of the bacterial masses the method of paddle wheel aeration was adopted with electric motor and stirrer. For a detailed account of the apparatus the reader is referred to the author's paper on "Studies in intensive bacterial oxidation" *(Journal, Indian Institute of Science, Vol. VI, Part VIII)*. The temperature of the liquid was kept at 25° C. to approach factory conditions. The acid formation developed up to 6·9 per cent. but suddenly at this point slimy growths of mucous bacteria made their appearance attaching themselves to the sides and internal projections in the barrel. These soon developed at the expense of the right organism and the result was a

diminution in acid content. Later experiments showed that this growth tended to oxidise the acetic acid to carbon dioxide. It is interesting here that this very often forms the pest of vinegar factories. Once they get these growths in, vinegar makers have to throw out a whole series of barrels and start the game afresh. The highest developed, most evolved and hence much feared of these growths is the so called *B. Xylinum.* It has been found that the remedy for these things lies in prevention rather than cure. If the temperature of the fermentation liquor is kept in the neighbourhood of 32° C. from the outset, those bacteria which grow best at that temperature and which again are the most active acetifiers are favoured at the expense of the mucous formations. This whole question of large-scale control boils down to encouraging the growth and activity of the right type of organism in the life struggle for predominance which is incessantly going on between the various other types which come in from infection in large scale work.

An experiment with the barrel using a heating coil (nichrome wire wound round two mica plates, these again being sandwiched between two plain mica plates the whole being held in a wooden frame) giving a temperature of 32° C. resulted in the production of nearly 9 per cent. acid. The process was then made continuous by withdrawing small quantities of the liquid and refilling with alcohol of the same strength. In this way an intensive oxidation effect has been got, and the barrel worked to its maximum capacity.

Incidentally several interesting observations have been made as to the toxicity of certain salts on the acetification process. These have important theoretical bearings and for an account of work on the subject, the reader is again referred to the author's paper mentioned above.

To conclude, if earnest attempts are made on the part of vinegar-makers to bring to bear on to large

scale work these conditions regarding purity of culture, air-supply, temperature-control and nutrient material with beneficial results, the author's labours will have been amply rewarded.

IV

DENITRIFICATION.

BY

Kotwal, Yashwant Nilkanth, B.A., B Sc. (Bom.)

GENERAL

DENITRIFICATION may be defined as the reduction of Nitrates by bacteria involving the evolution of nitrogen gas or oxides of nitrogen in soil, in sewage filter-beds, or in artificial media.

Plant nutrition has received close attention and the nitrogen question in connection therewith has been studied by many investigators, the soil chemist, the soil physicist and the soil bacteriologist, all spending much time, energy and intelligence in elucidating the "Denitrification" phase of the Nitrogen problem.

Since the agriculturist is more concerned when denitrification changes liberate free nitrogen or oxides of nitrogen (involving a loss of fertiliser material), a sound knowledge of the conditions favourable to denitrification is essential. The presence of denitrifying bacteria is the first essential condition. Naturally, having to deal with life, food must be the next consideration and so a supply of nitrates must be present under anaerobic conditions. Along with nitrates, however, a large amount of easily assimilable carbon compounds is found to be necessary. Neutral salts of certain organic acids are found to serve best and certain denitrifying bacteria are known to use even sulphur as a source of energy. A high temperature (45° C.) and a high moisture content (25 to 30 per cent.) of the soil favour the liberation of free nitrogen.

A historical survey of the literature on "Denitrification" reveals that the reduction of nitrates in presence of organic matter was observed as early as 1846, but, as with every bacterial change in the infancy of bacteriology, was regarded a chemical phenomenon. It was in 1846 that the "Chemical Theory" of denitrification was finally abandoned for the bacterial one, when the French Chemists, Gayon and Dupetit isolated and described two denitrifying organisms.

Much has been said about loss of nitrogen in the soils and though this side of the problem cannot be entirely ignored it seems a just remark to make that the economic significance of denitrification is overestimated and that under field conditions, denitrification is a factor of slight importance. Regarding farm-yard manure heaps, it is found that the losses of nitrogen are greater and proceed much more rapidly than in the soil and depend upon (1) nature of the manure (2) structure of the heap and (3) place of storage. Experiments at Rothamsted Station show that, in general, the loss of nitrogen compounds is rapid at first and falls off afterwards, cow manure showing a loss of 38 per cent. in 9 months' loose storage whilst in bullock dung for the same period the loss is 50 per cent.

Sir John Russell, Director of Rothamsted Experimental Station, has shown by means of a hermetically sealed apparatus that the loss of nitrogen in manure heaps, in part at least, is actually a gaseous loss. From the early history of denitrification there have been suggested three main hypotheses to account for this loss of nitrogen gas:

(a) Put forward by Wagner, was a purely reduction hypothesis, nitrate being decomposed in absence of air to nitrogen gas.

(b) The oxidation hypothesis, by which it was supposed that evolution of nitrogen took place in presence of oxygen and was the result of direct oxidation

or combustion of the nitrogen compounds to gaseous nitrogen. Reduction of a nitrate was not assumed.

(c) Alternate reduction and oxidation, nitrate being formed in presence of oxygen and reduced in the absence or deficiency of oxygen.

The exhaustive experiments of Sir John Russell show that loss of nitrogen only occurs under mixed aerobic and anaerobic conditions and this complex air requirement indicates an alternate oxidation and reduction as postulated in the third hypothesis.

The mechanism of denitrification, i. e., the actual way in which the nitrogen may be lost in the gaseous form is explained by William Hulme of Manchester, working under the guidance of Dr. Fowler as due to (1) Bacterial reduction, and (2) Enzymic reduction of nitrates. Examination of gases from media with and without nitrates showed 98 per cent. nitrogen gas from nitrate media, the remainder being carbon-dioxide: and 70 per cent. hydrogen and the remainder carbon dioxide from nitrate-free media. Thus the actual chemical agent by which the organism brings about the reduction is hydrogen. The media were tested for enzyme action and the nitrate-containing flasks did give a positive result. The mechanism of denitrification may therefore be represented as follows:—

$$C + H_2O + \text{organism} = CO_2 + H_2 + \ldots\ldots \text{ or}$$
$$CH_2 + O_2 + \text{organism} = CO_2 + H_2 + \ldots\ldots$$

where C represents the carbon of the nutrient medium.—

(1) $KNO_3 + E\ (\text{Enzyme}) = KNO_2 + EO$

(2) $EO + 2H = E + H_2O$

(3) $2KNO_2 + 5H + 2CO_2 = 2KHCO_3 + 2H_2O + N_2$

A view is also expressed that the liberation of nitrogen and carbon dioxide in a mixture by denitrifying bacteria, in a medium containing meat extract peptone, and nitrates is due to the reduction of nitrates

to nitrites, the interaction of the latter with amino-compounds in presence of acids generated by the fermentative action of the bacillus.

$$C_2H_5NH_2 + HNO_2 = C_2H_5OH + N_2 + H_2O.$$

EXPERIMENTAL

It has been claimed that the greatest loss of nitrogen coincides with the largest production of nitrites. Nitrites are an intermediate formation in the bacterial oxidation of nitrogenous organic matter and ammonium nitrite itself is an unstable substance; also, nitrous acid reacts easily with urea, amines or amino-acids under the ordinary conditions of the laboratory, with elimination of nitrogen according to the well-known general equation:—

$$HONO + R.OH_2 = R.OH + N_2 + H_2O$$

It has been commonly assumed that the amino compounds formed by the natural decomposition of proteins do actually react with nitrous acid produced during nitrification of the ammonium compounds also present.

The object of the following work given in brief summary was to discover whether such elimination of nitrogen occurred during the oxidation of organic nitrogenous matter when present in highly dilute solutions as in sewage.

PART I.

Experiments with Ammonium Nitrate.

(a) Time constant, temperature varying.

(b) Time varying, temperature constant.

(c) Action of carbon dioxide, both free and combined as bicarbonate.

(d) Action of various acids and urea.

(e) Action of sulphuretted hydrogen.

PART II.

Reaction between ferrous hydroxide or sulphide and ammonium or sodium nitrite or hydroxylamine.

PART III.

Possible reduction of ammonium nitrate by oxidisable substances.

Part I.—Ammonium nitrite was prepared from a cold saturated solution of silver nitrite and a fairly strong solution of ammonium chloride. The dilutions of ammonium nitrite used were 0·15 per cent. and below. It was found from (a) and (b) that:—

1. Ammonium nitrite in solutions from 0·15 per cent. to 0·0015 per cent. *i. e.*, 32·8 to 0·328 parts of nitrous nitrogen per 100,000 is completely stable when heated to 50° C. for a period of 6 hours continuously.

2. Ammonium nitrite in solutions from 0·014 per cent. downwards is also stable completely when heated continously for a period of 5 hours at the temperature of the boiling water (96° C. in Bangalore).

3. Ammonium nitrite solution of 0·16 per cent. undergoes decomposition at the temperature of boiling water, the decomposition being roughly proportional to the time of heating.

4. Since even 0·3 parts of nitrous nitrogen per 100,000 is a very rare occurrence in practice, ammonium nitrite is stable in solutions as occur in dilutions of sewage effluents and the loss of nitrogen therefore cannot be partly or wholly explained by the decomposition of ammonium nitrite itself, as might be expected from the generally assumed instability of this compound.

(5) It has been shown by Moore that on passing carbon dioxide through strong solutions of potassium nitrite, nitrous fumes are liberated. In order to test the effect of carbon dioxide on dilute solutions of ammonium nitrite solutions from 0·15 per cent. and downwards

were employed; in one case the carbon dioxoide was added as calcium bicarbonate and in another the free gas was introduced. It was seen from these experiments that:—

(1) Ammonium nitrite from 0·15 per cent. and downwards is not decomposed in presence of calcium bicarbonate when heated to 50° C. for a period of 5 hours.

(2) A current of free carbon dioxide bubbled continually for 5 hours through solutions of ammonium nitrite from 0·15 per cent. downwards at 50° C. has no effect on the solutions.

The obvious conclusion which follows from (1) and (2) is that the carbon dioxide either in septic tanks or filter beds does not seem to play any part in the losses of nitrogen.

(d) The experiments on interaction of nitrites urea and various acids were performed in an ordinary nitrometer. Acetic, amino-acetic, uric and hippuric acids, or water saturated with carbon dioxide did not liberate any nitrogen at all. For the purpose of ascertaining whether loss of nitrogen occured when a mixture of amino-acids (prepared by hydrolising glue with hydrochloric acid and subsequent removal of free hydrochloric acid as far as possible by distillation in vacuo) was brought in contact with a dilute solution of ammonium nitrite an experiment was tried in a modified form of apparatus which allowed the use of a greater volume of liquid. The apparatus is a bottle to which is attached the nitrometer and also a siphon tube. No evolution of nitrogen was observed during 12 hours or when 10 c.c. of N/10 sulphuric acid or water saturated with sulphuretted hydrogen was added.

It would seem, therefore, that in such dilute solutions as are likely to be met with in practice, no appreciable evolution of gas takes place through the interaction of nitrous acid and amino compounds. Just

as ammonium nitrite is stable in dilute solutions, so are seemingly the other possible combinations of the amino group and nitrous acid.

(e) Sulphuretted hydrogen is not infrequently produced by ultimate decomposition of the protein matter in sewage, and also by the action of carbon dioxide on sulphides of iron, which is often formed in sewage filter-beds, where there is defective aeration.

Qualitatively, it was found that if sulphuretted hydrogen is passed in or hydrogen sulphide saturated water is added to a neutral solution of sodium, ammonium or potassium nitrite, colloidal sulphur appeared almost immediately in strong solutions and after a time in the case of weak solutions. Hydrogen sulphide being a reducing agent, the appearance of sulphur was taken as a result of its reducing action on the nitrite, itself being oxidised to sulphur and water.

Quantitative experiments showed, however, that the nitrite was not reduced in the least. This was proved by removing all sulphide present by either lead carbonate or cadmium sulphate and determining the nitrite in the solution either calorimetrically by the Griess-Illosway method or by actual titration in the usual manner with permanganate. It was also incidentally observed that:—

(1) Light, oxygen either free or dissolved does not seem to have any connection with sulphur precipitation, the sulphur being precipitated with equal rapidity in darkness and in absence of oxygen.

(2) The precipitation of sulphur is dependent on the amount of nitrite, and

(3) If a large amount of nitrite is present (say 1 gram in 250 c.c.) in solution, the precipitated sulphur goes into solution after a time, forming a yellow coloured liquid like that of yellow ammonium sulphide.

Part II.—Wherever sewage matter is allowed to decompose in presence of soil or other material containing

iron, there is a tendency for a reducing action to take place and for ferrous compounds to be formed. It has been shown by Dunstan and Dymond that ferrous hydroxide, when allowed to act upon dissolved sodium nitrite, produces hyponitrite, nitrous oxide, or free nitrogen according to the conditions which obtained. They also indicate that hydroxylamine is decomposed by ferrous hydroxide.

It seemed of interest, therefore, to study the interaction between dilute solutions of nitrite and ferrous hydroxide or sulphide, either as a precipitate in mass or covering portions of material such as pumice in such a way as to imitate the conditions sometime occurring in bacterial filters.

The experiments were performed in glass towers with a gas delivery tube connected to a nitrometer or in conical flasks connected to a nitrometer. The towers were filled with pumice on which freshly precipitated ferrous oxide in varying amounts was poured, or in the interstices of which the hydroxide was precipitated. The nitrite solutions never exceeded 0·15 per cent. and were either poured in a lot to fill the tower to the neck or were admitted drop by drop in which case the air in the tower was replaced by carbon dioxide.

In the case of conical flask experiments, 75 c.c. capacity space was filled with varying amounts of ferrous hydroxide or sulphide and the remaining air removed as far as possible by the nitrometer so that it maintained the mercury at zero when the nitrometer was opened to the flask. By a blank experiment, the volume above the 75 c.c. mark was determined by filling the flask full to the neck, after the nitrometer had maintained its equilibrium point. Solutions in question were dropped from the funnel and after allowing 18 hours, the gas collected in the nitrometer was measured. In all these experiments no evolution of

nitrogen was observed. Ferrous sulphide also gave negative results.

In all the experiments of Part II. the amounts of nitrogen in solution that could have been liberated exceeded 50 c.c. of the gas at N.T.P.

Part III.— Qualitative experiments were made to see if ammonium nitrate when heated with oxidisable substances like sugar etc., would be reduced to ammonium nitrite. Ammonium nitrate solutions from one per cent. to 0·001 per cent. were heated at 50° C. for 6 hours, with corresponding solutions of glucose and starch, with extremely fine filter paper pulp and with the pulp while free carbon dioxide was bubbled through the reaction mixture. The solutions were tested from hour to hour for nitrite with Griess-Illosway re-agent, but in no case was the faintest trace of nitrite detected.

Ammonium nitrate and hydrogen sulphide showed no nitrite after the removal of H_2S, after a contact of 6 hours at the room temperature, nor is any sulphur deposited as in the case of ammouium Nitrite and H_2S, nor any free ammonia detected when sodium nitrate is kept mixed with hydrogen sulphide for over 48 hours.

The general conclusion of all these experiments is that evolution and consequent losses of nitrogen as gas taking place in nature or in the operations of agriculture and sewage purification due to purely chemical causes are negligible so far as the above reactions were investigated. This conclusion is somewhat unexpected, but accords with the results of Russell and Smith in their researches on the formation of nitrates in nature by purely chemical means.

V

FILAMENTOUS BACTERIA IN ACTIVATED SLUDGE.

BY

N. Swaminadhan, B.Sc.

THE Activated Sludge Process of sewage treatment has long been known to be one of intensive bacterial oxidation. It has been noticed by various observers that this process is checked from time to time by the development in the sludge of certain non-bacterial as well as some filamentous bacterial forms which bring about protracted settlement of the sludge, thus rendering it unsatisfactory. Whenever such a phenomenon happens the term 'bulking,' has been invariably applied to it. In an interesting paper on the activated sludge process, Ardern and Lockett (*Jour. Soc. Chem. Ind.*, 1923, 225 T), state that this phenomenon "results, as a rule, in the temporary loss of control of the activated sludge in circulation. Under these conditions, the volume of sludge present in the aeration chamber, as measured by volume after one hour's settlement, may increase in the course of twenty four hours to such an extent as to approximate to twice the original, and may continue to increase over a period of several days. Settlement is protracted, the separation of the sludge from the purified effluent is attended with some inconvenience, and the resultant effluent is liable to contain an unusual amount of solids in suspension. Microscopical examination of the sludge at such times has usually revealed a preponderance of certain types of higher organisms, such as, e. g., *Earchesium, Stentor,* and filamentous growths of the *Cladothrix* type thus indicating that the 'bulking' of the sludge is associated in some way with these protozoal and bacterial growths."

Researches in this direction have been conducted by the author with the experimental activated sludge plant at the Indian Institute of Science, Bangalore. The author comes to the conclusion that though the non-bacterial population are harmful to the bacteria causing purification of sewage the 'bulking' phenomenon is brought about mainly by some filamentous bacteria and the protozoal growths have nothing to do with it.

The activated sludge plant was brought into operation on the 10th July 1922, and was not pushed through as rapidly as might have been possible in order to study the biological phenomena occuring in the initial stages. The compressed air for agitating the sewage in the tanks was derived from a small Reavell Blower which delivered about 20 cubic feet of free air per minute. This amount of air supply is only half the quantity recommended by Messrs. Activated Sludge, Limited, for this particular plant.

Ever since the plant was put into operation, systematic analysis of the effluent and an extensive biological examination of the sludge have been conducted. The effluent was clear and contained very little suspended matter, but showed only traces of nitrite and nitrate for four or five weeks. The sludge on the other hand was characterised by an extraordinary development of a variety of animal life varying from minute protozoa to worms and insect larvae. The sludge itself was of a medium- brown colour and settled rapidly. These observations were continued for over a year and throughout that period the sludge, though settling quickly still contained organisms such as *Vorticella, Stylonichia, Philodina, Chironomus* larvae, etc.

In the middle of July, 1923, the sludge began to settle very slowly and the volume measured after an hour's settlement showed an unusually abnormal

increase. The sludge was gelatinous and sticky and the particles could be separated only with difficulty. Examination of this sludge under the microscope exhibited the presence of huge masses of filamentous matrix; there were very few higher organisms. The volume of sludge deposited after a one-hour period on the 31st August was 32 per cent., and the volume of the sludge measured after the same period of settlement on the 4th September was 60 per cent. Therefore it is obvious that the immediate cause of bulking of the sludge must be the filaments. It is quite improbable that so much sludge would have been built up during the course of 4 days and so great a quantity of sludge has never been built up in that time during the author's observations of the plant for over a year. The statement of Ardern and Lockett that the bulking of the sludge is associated in some way with the protozoal and bacterial growths is only partly true.

The filaments under the microscope appeared as long straight rods, very thin compared with their length, and non-motile. The chief aim of the author has been to isolate the filament and study the conditions under which it occurs in the activated sludge and to find a method of eradicating it. The filaments were cleared of extraneous matter by repeated washing and inoculated into sterile ferrous ammonium citrate tubes. No change was observed in the medium even after several days. This solution was then poured into a Petri dish and thickened with nutrient agar and incubated at 37°C. In this case a number of white filamentous colonies was observed. A permanent stain preparation of one of these colonies was found to appear as long, chainlike filaments, and a pure culture was obtained from this after a number of sub-cultures. This bacterium has the property of liquifying gelatine and produces a white, filmy gowth. It refused to grow on sewage sterilised by ultra-violet light. As high a temperature as 80° C. does not seem to affect it. It does not grow on nutrient

agar containing 0·2 per cent. acetic acid but 0·1 per cent. lactic acid does not affect it.

In the present paper, the author describes the procedure adopted for isolating the organism and does not claim to have succeeded in the attempt. The work is still in progress and it will be premature to offer any conclusion.

It was thought quite probable that these filaments may belong to the iron group or bacteria and following this line of thought certain laboratory experiments were carried out adding iron salts to the sludge.

Three narrow-mouthed graduated cylinders of two-metre capacity were supplied with equal volumes of mixed liquor collected from the ten aeration chambers. To one of the cylinders, containing the sludge, two parts of ferrous sulphate per 100,000 was added. To the second, the same amount of ferric chloride was added and the third was kept as a control. The cylinders were connected together in such a way that air could be bubbled through all of them. The volume of sludge deposited during a one-hour period in all the cylinders was noted after every 24 hours' aeration. The supernatent liquor was examined chemically for ammonia, nitrite and nitrate, and the sludge observed under the microscope for any biological forms. The liquor before aeration contained a large quantity of ammonia and after blowing air a gradual conversion first into nitrite and then into nitrate was noticed. The sludge contained numerous ciliates and filaments before aeration but afterwards a reduction in the filaments was noticed though the ciliates were unaffected. There was no marked difference in the flocculation of the sludge in the three cylinders. It is believed that this organism may be *Bacillus Subtiliformis* because of its occurrence chiefly in sewage and the similarity it offers to the above organism in more respects than one.

This suggestion is made provisionally subject to confirmation by further research. It is found that vigorous aeration breaks up the filaments and the sludge can be freed from these forms in this manner.

VI

A BIOCHEMICAL INVESTIGATION OF THE ACTION OF CERTAIN LOW FORMS OF VEGETABLE LIFE ON TEXTILE FABRICS.

BY

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MILDEW is the damage resulting from the growth of a class of low fungi which stain and sometimes tender textile fabrics. It has been the cause of immense losses to the Cotton manufacturers, especially for the tropical Markets, where the condition of temperature is most favourable to the growth of micro-organisms. In every case of mildew in cotton, it will be found as a main factor, that the cloth has been in a damp state. This condition, in the presence of the starch or flour contained in the size is all that is required for the favourable growth of fungi. A systematic investigation of mildews from a Biochemical standpoint was carried out at the Municipal College of Technology, Manchester, England.

I.

It has been shown that an infinitesimal quantity of good material, suitable to the requirements of micro-fungi is naturally present in all raw cottons, and if these are exposed to moist warm conditions for a prolonged period of time micro-fungi make their appearance. It has been observed that the micro-fungi first appear on the surface of individual fibres and send their mycelium right through the canal of the cotton hair. The following is a micro-photograph showing the penetration of mycelium through the cotton hair.

II.

Experiments were carried out with five different samples of fabrics, two of which were uniform in structure whilst the remaining three were average specimens of the tropical markets, representing three grades of sizing, light, medium and heavy. Mildew appeared in all the fabrics with the exception of one uniformly scoured fabric, after exposure to a moist warm condition for over 30 days at 22·2° C. It was also found that the growth of mildew is appoximately proportional to the quantity of foodstuffs present in the fabrics in the form of size-meterials.

The various colonies were selected from the mildewed portions of the fabrics by means of a sterile platinum needle under all possible sterile conditions, and were finally inoculated into the following media:— (1) Wort Gelatine (2) Nutrient Gelatine (3) Nutrient Starch Agar and (4) Glucose Gelatine. Mixed cultures were, however, obtained and a number of sub-cultures were made out of these different growths to isolate the organisms in a pure condition.

"*Aspergillus Niger*", "*Penicillium Glaucum*" and "*Mucor Mucedo*" were isolated from the mildewed fabrics. Along with the fungoid growths some small bacterial colonies were observed in three different cultures. A careful microscopical examination showed they contained some "rod shaped bacilli" of motile nature. Difficulty arose here in getting rid of the fungoid growths from these colonies. On Dr. Fowler's suggestion, the bacilli were sucessfully isolated in a pure condition by repeated sub-cultures on the nutrient gelatine medium without neutralising the organic acids (the acidity of 100 c. c. in terms of N. alkali 0·25 c. c.). The characteristic properties of these bacilli were further studied. Their behaviour towards various carbohydrates is very interesting. When inoculated

into pure cane-sugar solution, they could hydrolyse it into monosaccharoses, which were further acted upon by the organisms with the production of iodoform-producing bodies. Similar results were obtained when they were inoculated into pure monosaccharoses, e. g. dextrose or laevulose. These bacteria when inoculated into pure starch solution, could ferment it into iodoform producing bodies. The presence of nitrogenous matter seemed to increase the activity of the bacteria. The action of these bacteria on pure cellulose (acid washed Swedish filter paper) was tried, but the action was extremely slow. After a fortnight's incubation, the solution on analysis was found to give Fehling's reaction, as well producing iodoform with iodine and caustic soda. Also it was observed that the solution could give the iodoform reaction in the cold as well as with ammonia indicating the presence of acetone as a possible degradation product of cellulose.

The symbiotic action of these isolated bacteria and the various fungi, seems to have a great influence in the degradation of starch and cellulose, the main constituents of size and cotton respectively.

Tensile strengths of the fabrics (before and after mildew formation) were determined, which conclusively revealed the nature of deterioration of the fabrics due to the action of these low forms of vegetable life.

III.

Experiments were made to investigate the effect of sizing the yarns with different kinds of starches. It was observed that "*Tuber Starches*" were more susceptible to the action of micro-organisms than the "*Cereal Starches*", a fact of considerable interest.

Finally, preliminary experiments were made as to the possibility of using gaseous antiseptics in warehouses,

without actually using any antiseptic in the size ; the use of formaldehyde seemed very promising.

In conclusion, I should like to express my deep gratitude to my former Professor, Dr. Gilbert John Fowler, D. Sc., F.I.C. of the Indian Institute of Science, Bangalore, who personally went to Manchester and kindly arranged for my research work in the University in 1921, which enabled me to carry out biochemical investigation applied to textile industries.

My sincere thanks are also due to Professor Turner and Professor Grant of the College of Technology, Manchester, for the interest they have taken in the progress of the work.

VII

PRINCIPLES OF INTENSIVE NITRIFICATION

BY

Mriganka Bhushan Roy, B.Sc.

THE incessant bacterial oxidation of ammonium salts to nitrates occuring in soils, is one of fundamental importance in the economy of nature. Putrefaction would ensue and plant and animal life would be extinct, if the great nitrogen cycle were not completed by means of this process. Plant physiologists have recognised that nitrates provide plants with an ideal source of nitrogen and the biochemist has always considered the nitrifying power of a soil as one of the essential data for determining its fertility.

The discovery of the "Activated sludge" process of sewage disposal is in many ways an epoch-making event in the history of the conservation of nitrogen. Apart from providing the sanitary chemist, a means of converting offensive sewage into harmless and useful products, it has to be looked upon as essentially a process in which the oxidising faculties of micro-organisms have been developed to their maximum capacity. To the biochemist therefore, the process stands fundamentally as one of intensive bacterial oxidation.

The most outstanding among the practical applications of this method are the acetification of alcohol and the nitrification of ammonium salts and the present paper is devoted to an elucidation of the broad principles underlying the latter process at the same time indicating the limitations incidental to all such biochemical phenomena.

The oxidation of ammonium salts proceeds in two stages, each one of them being controlled by a specific

kind of bacterium. The first conversion of the salt into nitrite is brought about by *B. Nitrosomonas*, while the next stage of oxidation into nitrate is achieved by *B. Nitrobacter*. The celebrated Russian Bacteriologist Winogradsky, isolated them in pure culture and established this point. He also proved that these organisms are prototropic in so far as they utilise carbon from carbon dioxide of the atmosphere. The energy required to split the molecule of carbon dioxide and assimilate its carbon, is obtained from the combustion of ammonia to nitrates. Potassium, magnesium, phosphorus and sodium are necessary for bacterial development. The materials from which the bacteria build up their cells are compounds of the simplest character, carbon dioxide, ammonia and a few mineral salts and it may be remarked that a simpler synthesis of proteids is scarcely conceivable.

With a knowledge of the above main conditions of nitrification one has to consider methods by which they can be intensified. The ultimate result, increased oxidation of ammonium salt, can be achieved in two ways:

(1) an increase in the concentration of bacterial numbers representing an increase in the concentration of the oxidising substance, or

(2) an increase in the oxidising capacity of the same quantity of bacteria.

It is perhaps true that in practice the increased efficiency may be obtained in both ways. Considered physiologically, the former is a biological phenomenon whilst the latter is a constitutional one. Conditions favouring the multiplication of bacterial numbers are, optimum temperature, 37° C., a good supply of nutrient salts, an unfailing supply of the basic substance which continuously removes and neutralises the products of oxidation and efficient aeration while those which favour the latter function are stimulants which act catalytically.

VIII

UTILISATION OF REFUSE AS NITROGENOUS FERTILISERS.

BY

Raghunath Dattaji Rege, *B. A. (Hons.) B. Sc.*

INTRODCTION.

Though the Great War was the direct cause of widespread devastation and loss of life, true Scientists must admit that it brought certain compensation in its train. Leaving aside the consideration of the most efficient scientific appliances of war, which enabled Germany to fight successfully for the time being against such heavy odds, she could manage even to ward off the food scarcity, in spite of blockade, by the use of judicious fertilisers, thus raising four crops in a year instead of two as usual. In spite of Sir William Crook's threat of nitrogen famine pending the discovery of new sources of nitrogenous fertilisers, England remained quite unmoved, and mastery of the sea, due to the well equipped navy alone, enabled her to save herself with difficulty from starvation.

Thus it is, that recently the problem of nitrogen fertilisers has come to the forefront, and practical scientists are seriously grappling with it. Leaving aside the natural deposits of nitrate in Chile and the availability of ammonium compounds resulting as a bye-product of many chemical processes, there are two great sources of nitrogen as yet practically untouched. In the atmosphere, which consists approximately of 21 per cent. Oxygen and 79 per cent. nitrogen by volume, there is an inexhaustive source of nitrogen; and had plants been able to absorb it directly from air as is done with carbon dioxide, nitrogenous fertilisers would not

have attained such an importance. During recent years an enormous amount of work has been done on the conversion of this atmospheric nitrogen into nitrates and ammonium compounds, since these are the forms of combined nitrogen for which agriculture makes the largest demand. Had it not been for the consumption of much expensive energy, which renders the product a luxury to the farmer on account of its high price, the problem would have been comletely solved.

Industrial waste proudcts and demestic refuse furnish the second great source of nitrogenous fertilisers, and the cheap bacterial processes for converting them into good manure would substantially diminish the prices of fertilisers and tend to establish them on a commercially sound basis. This would solve one of the present difficulties confronting municipalities, for, owing to shortage of the fuel required for burning such vast amounts of refuse, the big Municipalities are abandoning the old method of incinerating the stuff and are seeking other avenues for the disposal of refuse. At last they have found its utilisation as a fertiliser as the best possible solution, being also a paying concern. Till now the London County Council, for instance, was spending one-seventh of the cost of all the municipal services omitting schools, police and relief of the poor, on refuse disposal. But with the slight progress which they are able to make with the help of scientists in turning some portion of it as fertiliser, they are recovering one-tenth of the cost.

COMMERCIAL POSSIBILITY OF REFUSE.

The commercial possibility of refuse, specially of garbage, is the recovery of fats and preparation of fertiliser, leaving aside the clinkers which can be used for brick making. The first-named is the ingredient representing the main value to be recovered. This is

done in the United States and some other scientifically advanced countries by either cooking under pressure with steam and pressing out liberated fats, or extracting grease with gasoline from dried stuff. Among those of fertiliser interest combined nitrogen has the greatest value. But due to the bulky nature of refuse and the lack of intelligent knowledge about its judicious application, its utilisation as manure is not so popular.

DIFFICULTIES.

The difficulties in achieving this object are also enormous. Firstly the composition of refuse is very variable. The refuse as a rule contains fallen leaves of different trees, street sweepings, straw and garbage, the last varying a great deal in composition. Garbage may be defined as the waste material arising from the Culinary department of a household or other establishment. It consists of large quantities of trimmings from green vegetables, the parings of fruits and vegetables, meat skins and bones, pieces of fat and egg-shells, containers of food materials, of paper, wood, tin, glass and portions of them. Thus it can be seen that the composition of refuse is likely to vary not only from place to place, but from season to season as well depending upon the seasonal fruits and vegetables. No general procedure is possible therefore in its conversion into fertilising material.

Secondly, nitrogen exists in a variety of forms while it is assimilable with benefit by plants in the form of nitrate only. Further some nitrogenous organic compounds are harmful to the plants and *Schreiner* has found that fertility or infertility of many soils depends upon the presence or absence of those. Therefore it is not only necessary for plants that nitrogen should exist in compounds soluble in the soil solution as is the case with phosphate and potash fertilisers, but in addition it should be present in certain definite forms,

Now, refuse being a conglomeration of different materials, does contain nitrogen in all kinds of compounds found in Nature, and to turn it into a good fertiliser all these harmful agencies must be eliminated by bacterial action. Thus it is necessary to study firstly the effect of these waste materials on the bacterial activities especially those of the ammonifying and nitrifying bacteria.

The want of a suitable analytical method for guaging the value of fertilisers is a serious handicap to progress in this line. There are three methods in vogue at present for this purpose viz., the chemical, the bacterio-chemical and the pot or plot culture. The application of all three methods is necessary for attaining a tolerably corrcet idea of the value of fertilisers and even then occasional disappointment awaits the farmer due to the individuality of the soil and climatic conditions. Out of these methods the chemical method is the most arbitrary; the other two can be credited in ascertaining the approximate quality of the fertilising meterial.

EXPERIMENTAL.

Till now wherever refuse is used as a fertiliser, it is at once thrown on the fields and allowed to rot or dumped in a pit which is closed completely to have semi-aerobic or anaerobic conditions for six months or so and then used as fertiliser. The first method is not only unfavourable, but sometimes positively harmful to plant growth depending upon the composition of refuse. *A. Lumière* has found for instance, by experiments with barley seeds, that undecomposed fallen leaves retard germination and growth of seeds, this being due to the soluble products of fresh leaves acting as a reducing agent, thus sbsorbing oxygen and depriving the seeds of a supply sufficient for germination.

By the second method this harmful effect is eliminated. But there are three disadvantages against it. First

in such a case, where there is so much of waste for disposal, time factor must be taken into consideration. In a big city there would be a shortage of waste land where so many pits can be had for dumping refuse, and it would be therefore necessary to evolve some other rapid method. Secondly, it is well known that under anaerobic conditions present in the pit, free ammonia is formed which during the transport of the stuff volatilizes out thus causing much loss of nitrogen. Thirdly, if the pits are not well arranged to drain away the excess water, much of soluble matter is leached out.

Some skirmishing experiments are therefore being conducted in the Bio-chemical Laboratory of the Indian Institute of Science to eliminate all those disadvantages viz., the harmful effect of the fresh stuff, the loss of ammonia by volatilization, leaching of soluble compounds and lastly the time factor also. For this purpose activated sludge seemed to be a good starting point. For the manure to be efficient, the complex nitrogenous compounds must be decomposed to amino-acids or ammonia, which in turn are to be converted to nitrates thus eliminating the harmful effect of some of these compounds and preparing the assimilable nutrition for plants. The activated sludge contains all the beneficial bacteria for these processes, and further it itself being a well-established manure, its addition would increase the value of other manures. Furthermore, as in these processes no putrefaction takes place, disagreeable smells are out of question. A subsidiary idea is to see whether by the addition of refuse the easy dewatering of the activated sludge is possible. In cold countries this is a problem receiving the attention of scientists; but till now they have not come to any satisfactory solution.

It is found that when a heap of refuse is watered with effluent from activated sludge tank, within two

months the stuff crumbles down to a fine powder and the water extract shows a dark-coloured liquid containing large quantities of 'Humin'. It was therefore thought to accelerate this process by obtaining refuse in a fine powder and aerating it with activated sludge. But experiments proved that after easily decomposable copmlex nitrogen is removed, even the fresh inoculant of vigorous bacteria of activated sludge would with difficulty act upon the proteids and after a month's aeration only 25 per cent. of the proteid nitrogen was available in solution. No doubt this soluble matter is a better plant nutrient than the effluent itself, but a large amount of nitrogen remaining in the solids would thus turn out useless. Moreover, this is impracticable on a large scale, there being the necessity of vast fields near the tank for the utilisation of this liquid manure.

To avoid all those difficulties, solid manures were prepared by the slow addition of refuse powder to the activated sludge, which was kept stirred. Incidentally the arrangement was such that aeration also took place. By this method the question of filtration was completely avoided as the stuff is a semi-solid mass which can be dried very easily and being less bulky, on account of its powdery form, is easily transported economically. But these optimistic aspirations received an unexpected check on analysis. The bacterio-chemical and pot-culture analysis of these manures showed that the ratio of N : C has a great effect on nitrification and consequently the crops of Ragi obtained from these manures could not stand comparison with that of activated sludge. In fact, it was found that the start was made at the top end of the ladder and this set-back was therefore quite natural.

During all those in vestigations our subsidiary object about the solution of the problem of filtration of activated sludge was dominating us subconsciously, and we had

therefore to oust it out completely from our thoughts. Reduction of the ratio of N: C no doubt increased activity of nitrifying organisms, but brought in its train the disadvantage of the immediate formation of soluble nitrogenous compounds specially nitrates which remained in the solution when filtered. On the other hand, drying the whole semi-liquid mass would occupy much time and heat-energy which would require to be supplied either by the sun or by fuel.

In the meanwhile Richards and Hutchinson's success in the preparation of a well-rotted manure from hay by the addition of some soluble ammonium compounds gave us an impetus to start our work on quite different lines. The emperical analytical methods so far evolved have proved that a manure to be of first grade must contain a high percentage of insoluble active nitrogen. In fact a good manure should not contain nitrate or soluble ammonium compounds in large quantities at the start, as during the interval of its application and utility to plants, some portion is either leached away or denitrified. But if the manure containing active insoluble nitrogen is applied at the time of the sowing of seeds as is usually done, the stuff is ammonified and nitrified by the time roots are developed sufficiently to take up the nutriment, thus leaving no time to misappropriation of these soluble products. So far the preliminary experiments have given great hopes of success. It is found that with a ratio of N: C as 1: 6, 0·44 percent. soluble nitrogen can be turned to insoluble active nitrogen within 24 hours, but if the stirring is continued for a day more it is again decomposed. This naturally leads to the reduction of the ratio of N: C by both the increase in the nitrogen content and decrease in the carbon one due to the bacterial action—a process beneficial to further nitrification. In their experiments with straw decomposition Richards and Hutchinson have shown that straw cannot take up more than 0·7 to 0·75 per cent.

ammoniacal nitrogen, the excess being wasted away either by volatilisation or some other method; and this process takes four weeks to be complete. By this method we think it possible to accelerate the process greatly, and thus absorb the soluble nitrogen wasted in the effluent from activated sludge. It is further suspected that the ratio of N:C has also got a hold on this reaction but at this stage nothing definite can be formulated. Attempts are also being made to prepare a special sludge by the slow addition of refuse and thus habituating the bacteria to this treatment. In this way it is hoped to raise the activity of bacteria to convert the soluble nitrogen to the insoluble active one.

In spite of this mild success we can without circumlocution admit that we are as yet groping in the dark. In spite of various patents advertised for the preparation of manure from refuse, so far the literature on this subject leads us to conclude that the progress in this line is full of briars and thorns requiring patient and careful investigation and we might well confess with Tennyson

"So runs my dream; but what am I?
An infant crying in the night;
An infant crying for the light;
And with no language but a cry.".

IX

ARTIFICIAL MANURES AND THEIR ROLE IN INDIAN AGRICULTURE.

BY

Tonse Venkappa Hegde. B.A.

PIONEERS in scientific agriculture have endeavoured to find out the elements taken up from the soil by plants during their growth for building up their tissues. Analyses of crops have shown that they contain mostly the elements, carbon, hydrogen, oxygen, nitrogen, phosphorus and potassium and traces of a few other elements. The atmosphere is able to supply the carbon; a few plants are able to get a portion of their nitrogen also from the air. The rest of the nourishment, a great deal of nitrogen and all the phosphorus and potash and minute quantities of many other elements are taken up by the plant roots from the soil. It is for making good this loss, that manures are applied to soils. Besides supplying the plants with necessary foods, manures also improve the texture of the soil and thus help the roots to spread underground easily. Another important effect of manures is that it keeps the soil in a fit biological condition for the growth of the plants.

In Europe and America, where agriculture like any other profession is carried on in a scientific manner, the natural manure consisting mostly of the animal and vegetable refuse of the farm has been supplemented to a great extent by manufactured products from the factory. While the general farm yard manure has its own use, the chemical manure has some decided advantages over the former. It is generally prepared in a concentrated

form and bulk for bulk contains greater manurial value and hence can bear the cost of transhipment to distances whereas the farm yard manure being bulky material cannot be used anywhere except in its immediate neighbourhood. Another important advantage of chemical manures is that being special manures containing some particular plant requirement, they can be applied with discretion according to the need of the particular soil or of the particular crop.

Artificial manures can be classified mainly into three classes according to the predominant plant food which it is intended to supply. (1) Nitrogenous. (2) Phosphatic. and (3) Potash. We have also mixed fertilisers which contain various quantities of one or more of the above three constituents.

The most important nitrogenous manures are, ammonium sulphate a by-product in the manufacture of coke, sodium nitrate or Chili salt-petre a mineral from South America and calcium cyanamide, a product obtained by making atmospheric nitrogen combined with calcium carbide. Potassium nitrate is also a very good manure but its comparatively high cost and its being used for many other industrial purposes preclude its being extensively used as a cheap manure. Most of these nitrogenous manure are very soluble and hence are readily available for plants. A general tendency of fertilisers of this group is to stimulate the growth of plants especially the green foliage and as a result the ripening process is delayed. Fish guano, refuse of fish industry, dried blood and slaughter-house refuse can all be included under this class of fertilisers though the nitrogen contained in these is mostly in the organic form.

Bone meal, basic slag, superphosphate and other mineral phosphates are the most important of manures belonging to the phosphorus group of fertilisers. Bones

are degreased and sometimes glue also is extracted. The residue after this extraction is powdered and is used as manure. This contains as high as 40 per cent. of tricalcium phosphate and 4 to 5 per cent. of nitrogen. Basic slag is a by-product of the steel industry, and contains about 40 per cent. of tricalcinm phosphate. It also contains a large quantity of lime which has very important manurial value. Deposits of mineral phosphates which serve as manures are found in North America and Australia, the best quality coming from Florida containing as high as 75 to 80 per cent. of tricalcium phosphate. These, though slightly less soluble than basic slag can de directly applied to the soil. Often they are treated with sulphuric acid and converted into superphosphates, which are used as such or mixed with lime and converted into basic superphosphates. These manures are specially valuable for development of roots. Phosphates also hasten the ripening process.

The world's supply of potassic manures came almost wholly from the output of Stassfurt deposits from whence they were supplied either as Kainite or as double sulphate of potassium magnesium or Carnalite or even as crude potassium chloride. Wood ashes contain an appreciable percentage of potassium salts and hence have been used as manures from time immemorial. This class of manures is very important, for the development of starch in plants and fruits.

There are a few other substances, which, though they do not belong to any one of the above groups, yet are more or less indispensible for the growth of plants. Lime and sodium chloride may be mentioned under this head. Their precise action is not properly understood, but their action as very important soil improvers has been demonstrated beyond doubt. It is believed that lime removes soil acidity and improves the soil textures. Sodium chloride is supposed to enter

into double decomposition with some insoluble compound forming as a result soluble and hence available plant food.

It remains to be seen how far these products influence the Indian farmer. Of the industries in India, agriculture is by far the most important, two-thirds of her three hundred and odd millions make their living by it. How then is this basic industry of their vast land carried on? With the exception of crushed bones and fish manure (which, however, is restricted mostly to the west Coast of the Madras Presidency), there is hardly any other artificial manure worth mentioning. When we find how much even of this the Indian farmer uses for the improvement of his land, the sad plight of Indian agriculture becomes evident. Excepting a small portion of this used in the plantations of tea and rubber mostly by European planters, the bulk of this is exported to outside places. Statistics show that the country exports yearly about fifteen millions of rupees worth of manure. This annual export of so much plant food in a country whose only boast is her agriculture and its produce represents a decided loss to the country.

Lack of knowledge among the actual farmers about the benefits accruing from the use of artificial fertilisers, want of cheap sources of such manure and the gross poverty of the average Indian farmer may be mentioned as some of the causes that have led to such a condition. It remains for those who have the welfare of the Indian farmer at heart, to improve the existing conditions by:—

(1) Educating the farmer and his children.

(2) By supplying him with cheap fertilisers and

(3) By demonstrating to him the advisability of using a particular artificial product for a particular crop.

It is to be hoped that the industrial wastes of the factories and the sewage and filth of all towns and cities will be utilised in the best way so as to conserve their fertilising value. Then instead of becoming a nuisance they will solve to a great extent the problem of cheap manure supply and will add considerably to the wealth of the country.

X

GUM ENZYMES.

BY

Mangesh Anant Malandkar, M. A. M. Sc.

OBSERVATION of the two phenomena viz., the surface blackening of the gum mucilage, obtained from the gum-oleo-resin of *Boswellia Serrata* by the autoclave process, as well as that of the gum obtained by extraction with alcohol, on exposure to air, led to the examination of *Boswellia* and other gums for the presence of enzymes.

This blackening was supposed to be possible due to the action of an oxidising ferment as in the case of Japanese gum lac (juice of *Rhus Vernicifera*), although in the first case the idea looked hardly probable, considering the high temperature to which the gum was subjected in its preparation. The cause of this particular blackening on further investigation, was found to be, partly at any rate, fungoid infection.

The idea as to the probable presence of an oxidizing enzyme in the *Boswellia* gum, led to the examination of the extracted gum for its presence. The gum solution was found to give a blue colour with guaiacum tincture alone, the most general reagent for detecting the presence of an oxidase, thus showing that the gum contains an oxidase.

The literature on gum enzymes is meagre. No reference to any work done on the enzymes of the gum from *Boswellia Serrata* could be found. Nor are there any comparative data on the enzymic activity of simple gums such as gum arabic and gums from gum-oleo-resins. It was, therefore, thought worthwhile to study the enzymes of *Boswellia* gum and also those of gum myrrh (which is a gum from gum oleo-resin) and other simple

gums available with a view to comparing the enzymic activities of these gums.

With regard to gum enzymes there seems to be a great controversy on the question whether sugar is produced by them from starch paste or not. According to Weisner and Grafe the gum enzymes are capable of converting starch paste into dextrin but not into sugar. Reinitzer, on the other hand, found at least in the case of acacia gums that they could convert starch paste into dextrin and maltose. Whether this diastatic enzyme is identical in its action on starch paste with that occurring in malt i. e., whether it carries the conversion of the starch to the same extent as malt diastase, is not known. It is assumed by many that gums owe their origin to an enzyme capable of dissolving celluloses and hemicelluloses and converting them into gums. This assumption is rather peculiar as no experimental proof has yet been brought forward in support of it. The reason given for its absence in gums is that it may be very labile and so may have become altered in the gums already exuded from trees. No attempts seem to have been made to ascertain the part which is played by enzymes in the formation of gums. Attempt, therefore is also made to study this important question as far as possible. The following gums were examined:

1. *Boswellia Serrata* Gum
2. Myrrh
3. Gum arabic varieties A and B.
4. Gum from *Cochlospermum Gossypium.*
5. Gum from *Mongia Pterigospermum.*
6. Gum Kino from *Butea Frondosa.*
7. Two gums sent from Dehra Dun and described as *Sterculia.*

Of these, *Boswellia* and myrrh gums represent gums from gum-oleo-resins. The two varieties A and B represent gums of the arabic kind. Gum No. 4 is

characterised by its property of constantly giving off acetic acid recognised by its strong smell. The gums *Butea* and *Moninga* are characterised by their containing a large amount of tannin as is shown by their giving a blue black coloration with ferric chloride.

EXPERIMENTAL.

Preliminary experiments showed that all the gums except the first three were very feebly active as regards the enzymic activity. Only the first three viz., *Boswellia,* myrrh and gum arabic, therefore, were examined in more detail. It should be noted that in all cases where the presence of bacteria might affect the results, the reactions were carried out in a medium of saturated thymol water.

In the case of *Boswellia* and myrrh gums, the oleo-resin accompanying the gums was removed by dissolving it in the cold with 90 per cent. alcohol. The bigger lumps, after almost all the oleo-resin was dissolved, were crushed to powder and the powder shaken with alcohol to remove any oleo-resin that might be present. In this way a white powder in the case of *Boswellia* gum and a grey one in the case of myrrh were obtained.

Gum arabic was divided into two protions according to the colour of the lumps, the brownish lumps being termed "Gum Arabic A" and the white ones "Gum Arabic B".

Qualitative tests with a two per cent. tincture of guaiacum (two gm. guaiacum in 100 c. c. of 85 per cent. alcohol) freshly prepared and boiled with a little animal charcoal for about 10 minutes, to destroy traces of peroxides found to be present in the freshly prepared tincture, showed the presence of an oxidase in the case of *Boswellia* gum, myrrh and gum arabic A but not in the case of gum arabic B which became blue only after the addition of hydrogen peroxide. The intensity of reaction shown by *Boswellia* and myrrh gums was

practically the same while that in the case of gum arabic was much less.

On account of the too great readiness with which guaiacum undergoes oxidation, however, developing the characteristic blue colour, and also because blood and other oxidising bodies other than ferments yield the guaiacum test, more definite tests were tried. Of the numerous tests described in which phenols and amino-phenols are oxidised to bodies possessing various colours, these were employed viz,, *a*-naphthol, p-phenylene diamine and Spitzer's reagent. All these tests gave results resembling those of guaiacum. The production of colour was accelerated by the addition of hydrogen peroxide, which points to the existence of a peroxidase.

Instead of depending on the qualitative tests only, however, it was thought necessary to try quantitative tests in order to arrive at some definite quantitative difference in the oxidase activity of the gums. Two methods were tried, one manometric and the other gravimetric.

The first was carried out by a modification of Bunzel's method. According to this the amount of oxygen is measured by means of the lowering of pressure as measured by a monometer. A known amount of the substance (enzyme) in solution is allowed to act on a known amount of pyrogallol also in solution in the presence of air. The oxygen is transferred from the air to the pyrogallol by the enzyme. An equivalent quantity of carbon dioxide is evolved which is absorbed by a suitable reagent and the diminution of the pressure noted.

The modification consisted in the use of a Schrötter's apparatus instead of a Bunzel's one, in the use of soda-lime to absorb the carbon dioxide evolved instead of sodium hydroxide and hand shaking from time to time instead of a shaking arrangement in the air thermostat. Potatoe juice was used for comparison with the results obtained by Bunzel.

The variation in result from those obtained by Bunzel in the case of potatoe juice are worth mentioning. Bunzel's results show that the reaction came to an end in about two hours and a quarter while the present work showed that the reaction was slow and did not come to a stand still even after fortyfour hours. Edal Behram's results (using the same results as the one adopted here) with the oxidase of *Lantana Camara* also showed that the reaction is slow and comes to an end after about 40 hours.

According to the second *i. e.*, gravimetric method equal amount of the different gums were allowed to act on the same amount of pyrogallol in solotion, for a definite time under identical conditions and the purpurogallin formed was weighed. The substance produced was proved to be purpurogallin.

The results show that the oxidase activity of *Boswellia* and myrrh gums is not much greater than those of gum arabic A.

It has been recently shown by Onslow that the tissues and tissue-extracts of those plants which blacken on injury and give a direct oxidase reaction i. e., give a blue colour with guaiacum tincture alone, contain some substance characteristic of the catechol grouping in addition to a peroxidase (whlch develops a blue colour with guaiacum tincture on the addition of $H_2 O_2$); other plants which do not blacken on injury and do not give a direct reaction are devoid of substances with the catechol grouping. There is evidence to show that such substances occur in *Boswellia* and myrrh gums although they are prsent in very small amounts in the extracted gum lumps. They could not however be detected in gum arabic A.

There is some controversy as to whether nitrogen could be detected in gums by the process. It was therefore decided to investigate this point and also to

determine nitrogen quantitatively with a view to ascertaining if any difference exists in the gums as regards their nitrogen content.

Nitrogen could be detected in *Boswellia* myrrh and arabic A. gums by the method of Lassaigne as well as that of Will and Vanutrapp. In the case of the last gum, however, the ammonia evolved, when tested according to the method of Will and Vanutrapp, canot be detected with litmus paper owing to the highly acidic nature of the fumes and the small amount of ammonia. It can however be detected by placing a piece of filter paper moistened with Nessler's solution at the mouth of the tube.

Nitrogen was estimated according to Kjeldahl's method. *Boswellia* contains 3·03 per cent., myrrh 3·02 per cent. and gum arabic (A) 0·16 per cent. nitrogen calculated on the dry weight of the material. Bertrand found that in the case of his laccase the presence of manganese is all important. He found for example that the activity of the ferment is directly proportional to the amount of the metal present but whether manganese is essential for all oxidase action is uncertain for Bach states that he has prepared a tyrosinase which oxidises tyrosine in the absence of manganese. In order to decide therefore, whether the oxidase activity is proportional to the amount of manganese present, manganese was quantitatively determined in the three gums *Boswellia,* myrrh and arabic A.

The estimation was carried out according to the method of Berkeley as it was found to give results of a high degree of accuracy for quantities of manganese as small as 0·01 mg. of manganese when tried on standard permanganate solutions. The percentage of manganese in the dry gum was found to be 0·0095 in gum *Boswellia,* 0·0115 in gum myrrh and 0·0109 in gum arabic A. The results therefore point to the fact that in the case of

these gums at any rate, the oxidase activity does not depend on the amount of manganese present.

Tyrosinase, invertase and maltase were not found in the three gums. Enzymes capable of dissolving cellulose and hemicellulose, were tested for macrocheically as well as micro-chemically, but could not be detected in the three gums mentioned above; nor could they be detected in freshly exuded gums or in the extracts of plant parts from which the gums were observed to exude. It appears highly doubtful, therefore, whether the formation of gums is in any way dependent on the activity of enzymes capable of dissolving cellulose and hemicellulose.

The diastatic activities of the gums were tested by their action on starch paste (Lintner's soluble starch). The products of the hydrolysis of starch were found to be dextrin and maltose. In order to arrive at some quantitative difference in the gums as regards their diastatic activity, the diastatic power was determined by the method of Lintner as well as that of Ling which is a modification of the former method. The diastatic power according to the latter method, was found to be 3·7 degrees in the case of *Boswellia* gum, 3·6 degrees in the case of myrrh gum and less than one degree in the case of gum arabic A. Determinations of sugar formed, calculated as maltose according to Bertrand's modification of Fehling's method, by allowing equal quantities of the gums to act on equal quantities of starch paste under identical conditions at 45° C. showed similar results.

This diastase of *Boswellia* and myrrh gums differs from that of malt in the fact that when the copper reduction corresponds to about 50 gms. of maltose per 100 gms. of starch the conversion becomes so slow that for all practical purposes it can be said that a state of equilibrium is reached,

As regards their behaviour towards heat these gum enzymes appear to resemble other enzymes. Their enzymic activities are destroyed by boiling their solutions for a few minutes or by the addition of some sulphuric acid. The action of heat upon them in organic solvents is less marked than in aqueous solutions. They show great resistence to heat in the driest condition as the heating of the gums in the solid state in the steam oven for 18 hours, only brings about a diminution in the enzymic activities but does not altogether destroy it.

As regards the role of enzymes present in gums in their formation, various possibilities suggested themselves. Croft Hill's work on the formation of a disaccharide by the action of maltase on a concentrated solution of dextrose, brought out the essential facts that while in dilute solutions there was a breaking down of larger into smaller molecules, in concentrated solutions, there was a building up or synthesis of the simpler molecules into the more complex. Considered in this light, gums may be regarded as originating from a condensation of simpler substances like sugars probably on the surface of some colloid by the agency of a specific ferment. Conversely, the gum enzyme acting upon a dilute solution, might be expected to break down the complex gum molecule into simpler substances like sugars. The presence of an excess of tannin in gum-producing plants such as Acacia, suggested the possibility that tannin might be in some way connected with the formation of gums. The two points were therefore investigated.

The gum enzymes were found to have no action on gums. They were, however, found to act on tannins, converting them, into non-tannins. The definite nature of the product formed is not yet determined. Even extracts of plant parts from which the gum is observed to exude, contain some enzyme which brings about

in the tannins contained in them, changes similar to those of enzymes found in gums.

CONCLUSIONS.

1. The gums examined viz., *Boswellia*, myrrh and arabic contain oxidising and diastatic enzymes, those in gums from gum-oleo-resins being much stronger than those in simpler gums.

2. The gums do not contain tyrosinase, maltase, invertase or enzymes capable of dissolving cellulose and hemicellulose.

3. The activity of the oxidising enzymes has no relation to the amount of manganese present.

4. The gum-enzymes act on tannins converting them into non-tannins. This conversion may be an intermediate stage in the formation of gums.

XI
STUDIES IN THE NUTRITION OF THE LAC INSECT.

BY

M. Sreenivasaya,

PRELIMINARY.

THE nutrition of the Lac Insect is one of great complexity, involving as it does a system of constituents and forces, quantitative determination of which is extremely difficult. The occurrence of a yeast-like fungus in the plasma of the insect and bacteria in its intestines, further complicates the investigation. The small size of the insect embodying its various minute organs of vital activity, renders their dissection, so necessary for microchemical studies, a task of great delicacy and high manipulative skill.

The problem is also one of great diversity and fundamental importance, since, on the one hand, it presents various aspects of study and on the other, the successful prosecution of the industry mainly depends upon an unfailing and balanced supply of nutrients to the insects. An inadequacy or a deficiency of some essential constituents results in an emaciated growth of the insect, a poor secretion of resin, a low reproductive activity and often in heavy internal parasitism, to the total extermination of the whole colony.

I
THE NUTRITION OF THE INSECT CONSIDERED AS A CAPILLARY PHENOMENON.

Like a true parasite the insect cannot go through its life-cycle without the aid of its host. It deflects in its own favour not only a part of the nutrition

of the host but also a part of its energy to draw its food. There is evidence to show that the mechanism by which the insect feeds is one of capillary rise through the fine bore of its proboscis, which is controlled by the molecular forces in the plant. Seasonal variation of the pressure of the plant sap, therefore directly influences the capillary supply of nutrition to the insect and as a result, the insect is not fed at the same rate and to the same degree in all seasons of the year. This fact will serve to explain the dissimilarity and fluctuation of lac crops which are harvested thrice every thirteen months (in Mysore).

The functional activities of the insect are accordingly modified to suit the inevitable seasonal phenomena. The reproductive and secretory activities are high during the rains while both of them are at a low ebb during the following winter. When the pressure of the plant sap rises during the rains, the host is able to maintain a greater number of larvae than it does during the winter season when the flow of the sap is practically dormant. This observation is of great importance in practical lac culture, since it emphasises the need for exercising great care and sound judgment in controlling the larval settlement of shoots, in different seasons of the year.

The nutritional value of a host should be based not only on the merits a proximate analysis of its chemical constituents, but also on some physico-chemical data which give a measure of the flow of the plant sap, thus determining their true availability. In other words one has to determine not only its potential but also its dynamic value. An infected host-plant may be compared to a circulatory system the root representing the pulsating heart, the shoots the conducting arteries and the proboscides of insects, its capillary ramifications.

Both direct and indirect evidence point to the conclusion that the insect draws its nutrition mostly from the cambial region, although it should be acceded in virtue of the tenderness of the shoot, that osmotic translocation of nutrients takes place from other parts of the section. Girdling experiments with lac bearing shoots conclusively proved this point.

Once the larvae colonise a main shoot and begin to draw their nutrition, the flow of the sap is established to the region of attack. If by chance the side branches of the shoot happen to get infected later, by another batch of larvae, a great percentage of them, mostly towards their terminals, die of starvation, the flow of the sap not being available to them and the pulsation of the conducting vessels not being sufficiently powerful to force nutrition through their capillaries. Pinching the terminals of infected shoots, results in thick incrustations and healthy growths of lac, since the forward flow of sap is arrested and diverted in favour of the latterly situated larvae.

Practical culture experiments have shown that it is more economical to maintain insects on inclined shoots, both from the view point of resin-production, and larval development. It is better to have a few well-fed colonies than a great number of them maintained at the critical point of starvation. In the case of vertical shoots which are colonised all round by the insect, the growth of lac are comparatively poor. In the case of inclined shoots, the insect colonising their lower portions, gets its food with ease and in abundance, since the capillary flow is aided by gravity. When cuts or incisions are made on the lower portions of an inclined shoot, they exude gums or resins as the case may be, while the upper portions of the same shoot respond but slightly to similar injuries. This experimental evidence is in support of the above hypothesis.

II.
THE NECESSITY FOR A PHYSIOLOGICALLY BALANCED FOOD.

Physiologists have recognised the importance of an adequate and balanced nutrition to organisms for their all round development. The five main functional activities of the insect, maintenance, growth, secretion, excretion and reproduction, should proceed harmoniously for the success of the organism as a whole, the promotion of each one of the activities depending largely upon the presence of some special constituent in the food. Field experiments on various hosts and an analysis of their constituents taken together, show that a particular activity is specially stimulated by the presence of a specific constituent. The insect needs for its maintenance a good supply of nitrogen and carbohydrates and special protein requirements for its vigorous growth. The secretory activity is a direct function of the alcohol soluble resins while the inorganic constituents, chiefly phosphorus and calcium, control the reproductive activity. Abnormalities ensue as a result of unbalanced nutrition and the ideal host-plant is one which promotes harmoniously all the functions of the insect.

III.
NUTRITION CONSIDERED FROM THE PRACTICAL POINT OF VIEW.

From the practical standpoint one has to consider nutrition as a means through which the vital energy of the insect is utilised for the production of materials of economical value. Considered in this light, one immediately thinks of exploiting the secretory activity of the insect for obtaining lac, its excretory activity to conserve honey through the agency of domesticated bees, its reproductive activity to generate verile and resistant broods in abundance for the retention and extension of the industry.

The food which is provided by some host plants like *Butea frondosa*, is poor so far as the resin producing efficiency is concerned. Insects growing on them yield poor and thin incrustations while they build up comparatively thick growths of lac on *S. trijuga* and *S. talura*. While their reproductive activity is great when propogated on *Shorea talura*, it cannot be continued for more than few generations on *Butea frondosa*, *Acacia arabica*, and *Pithecalobium saman*. The generations successively degenerate to extinction.

Factors which promote the two activities of secretion and reproduction do not necessarily combine one and the same host-plant. *Acacia farnesiana*, although an excellent host for generating virile broods, is a poor resin producer. The virility of a *Shorea* brood has been kept upon this host to its eighth generation with remarkable success. This plant has provided us with an ideal host for brood farms and experimental work.

IV.

NUTRITION AND THE CONTROL OF THE SEX RATIO.

The proportion of males to females in a brood is a factor of vital importance to the practical lac-culturist, because, it is only the female that builds up a maximum amount of resin. The male contributes but an insignificant quantity of resin to the general incrustation of lac. A preponderance of males would mean a poor crop to the cultivator whilst that of the other sex would result in heavy crops.

Recent experiments with lac bearing *Acacia farnesiana* plants cultured in different nutrient solutions, has thrown some light on this important question. The detailed results will be incorporated in one of the Journals of the Indian Institute of Science. Field experiments have shown that the sex ratio is controlled

by the seasonal variations of temperature, humidity and moisture supply. Dry seasons and high temperatures, tend towards the creation of females while abundant moisture supply results in a preponderence of males. Calcium favours the production of males while phosphorus favours the determination of the female sex. Mothers developing during the rains yield a brood with a preponderating male progeny while those fed during the following winter period, give rise to broods possessing a larger percentage of females, so that, the following summer crop is comparatively better than the previous winter crop. During winter the mothers are fed more on fats than on carbohydrates while during the monsoons they are fed with a food containing a small percentage of fat. Fatty nutrition therefore appears to favour the determination of the female sex.

In the case of some wild species of lac insects, *L. Communis*, the variations of the sex ratio in response to nutrition and season is very marked and further the species can be propogated on *Acacia farnesiana* quite successfully. For these two reasons, the author has adopted this species of insect for his further experimental studies of this problem.

V.

NUTRITION AND IMMUNITY FROM INTERNAL PARASITISM.

A whole group of chalcid flies attack the lac insect during its life-history, some in its younger stages, others in the later stages of its activity. An exhaustive study of these internal parasites has been pursued by Mr. Mahdihassan who has established the interesting specificity of these flies to each species of lac insect, analogous to the association of specific bacteria with each kind of seed, established by the author some years ago and now being pursued by Miss Christie

in the Bio-Chemical Laboratories of the Indian Institute of Science.

Experiments with infected *Acacia* plants and pot-cultures with *Cajanus indicus*, showed that starvation or a deficiency in certain essential elements brings about the attack by these chalcids. Calcium deficiency for example renders the insect particularly susceptible to this kind of parasitism. The winter crop suffers from parasitisation, since the insects are not fed properly due to the prevalent scarcity of nutrition.

The adult chalcid fly lays its eggs in body cavity of the lac insect, where they develop as parasites and become free and perfect imagoes. One wonders how they are able to subsist in their host, possessing immunity to its fluids and phagocytes which digest or destroy the foreign organisms which succeed in finding their way there. Here we are faced with the whole problem of immunity. Evidently calcium, in some way, imparts immunity to the lac insect and the characteristic disappearance of yeasts from parasitised insects, leads us to suspect that in healthy ones, the yeasts play the role of phagocytes in disintegrating the parasitic eggs which may be laid in the deep-seated organs of the lac insect.

XII

ON THE SIGNIFICANCE OF SOME CONSTITUENTS OF LACS.

C. Rama Somayajulu, B. A.

I.

ON examining closely the several kinds of lac, the differences in character between the various samples, are impressive. In order to be able to form an idea as to whether such differences are superficial or deep rooted, analytical data of different kinds of lac would be most valuable. An attempt at such analysis was undertaken by the author, and in this paper some broad conclusions which seem to be possible from the analysis of about twenty-five samples obtained from different localities and at different seasons, will be indicated.

In a work of this kind, three fundamentals which tend to influence the formation of lac, should be distinctly kept in view; the genus of the insect, the species of the tree on which lac is propagated and the season in which a particular sample is grown. The importance of these factors can be realised if attention is paid to the two theories which have been put forward to explain lac formation. Should the view that lac is a waste-product, excreted by the insect from unassimilable matter of the plant-juice on which it feeds, be correct, different species of trees ought largely to control the nature of the lac grown on them. If, on the other hand the hypothesis that lac is a physiological secretion product of normal metabolism, out of raw materials obtained from the sap of the juice, the physiology and life history of the genus of the insect, has not a little bearing on the nature of the product. Besides, the composition of the

sap of a plant is liable to differ with seasonal variation. In the selection of samples for analysis, therefore due regard was paid to these factors.

II.

The composition of lac as hitherto studied by workers in this field may be defined as follows :— Waxes, some higher alcohols extractable by petrol-ether, some colouring-matter, fatty acids, soluble in ether, and resins removable by 96 per cent. alcohol, the lac-dye found in the insect plasma and the eggs and the honey-dew excreted out in the course of lac secretion. As the two last-named are not direct components of lac, attention was not paid to them in the course of our work ; the other products were obtained by successive extractions of different kinds of stick and seed lacs with petrol-ether, ether and alcohol.

Besides ascertaining the percentage of each extract, attempts were made at the determination of some constants like the saponification value, the iodine value, the acid value, melting point and refractive index. In the case of the ethereal and alcoholic extracts, the refractive index was not determined on account of the high colour of the extracts. Determination of the melting point and iodine value also was not made in the alcoholic extract as lac resin does not give a transition point and in fact never melts to a fluid, whilst the utility of the iodine number in the case of resins is still doubted and the reliability of results is a matter of controversy. As none of the extracts consists of a simple substance obtainable in a pure state, the absolute value of any one of these constants would be small, but all taken together would certainly aid one in forming an idea as to the variation existing in the proportion and nature of the components forming the extract. As this work was chiefly aimed at a comparative study, it was not thought worth while going into greater details.

Since in many cases the quantities of material for research would not be large, and since in most biochemical problems we expect to deal with only small quantities in order not to interfere with the living agencies at work, some micro-analytical methods are naturally to be sought. Methods were used, therefore, based upon the work of Gills and Simms *(Jour. Ind. Eng. Chem. 1921)* who tried to determine the saponification and iodine value of some oils but largely extended and modified to render them adaptable to fats, waxes und resins.

For fear of digression, I do not propose to deal with all the details of these methods, an account of which will shortly be submitted for publication in the Institute Journal. As little as 1 to 2 grams of lac would suffice for the whole analysis indicated above; for iodine value determinations 10 milligrams, and for saponification and acid values 15 milligrams, would be more than ample. The results obtained were fairly consistent and sharp.

III

The work is still in progress, but some interesting conclusions could safely be ventured from what has been accomplished. One very striking feature which emerges is the large divergence in saponification values of stick-lac as distinct from seed-lac. In the former case it is as much as 150-170, whereas in the latter it never exceeds 85. This fact is not inconsistent as a close examination of stick-lac reveals white wax-pouches and insect body-remains which are completely removed by washing. An analysis of the wax-pouches would be interesting in itself and has been taken in hand; but an independent comparative examination of various stick-lacs and seed-lacs shows that there exists some difference in the constituents of various samples. This differenc is not very marked where only the plants and seasons are varied, other conditions being kept constant, but is more appreciable where there is

a variation of the genus of the lac-insect. This conclusion is strongly and irresistibly confirmed in the case of *Tachardia Minuta* where the percentage of matter extractable by the solvents employed in the analyis is very small compared to the unexctractable consituents. Experiments carried out with this insoluble portion point to a large amount of residual carbohyrates. This is quite in conformity with the observations made here in following the formation of different lacs on an experimental host, *Acacia farnesina*, cultured in nutrient solutions. The absence of ants due to the non-excretion of honey-dew from *Tachardia Minuta* leads one to infer that this insect has deficient digestive and secretory systems. More work is being done in that direction.

The results then may be interpreted to mean that the nature of the insect more than anything elseplays a very great part in the formation of lac, thus supporting the view that lac is a secretory product. Some more very interesting conclusions are anticipated by which we might approach more closely to an understanding of the genesis of lac in relation to the physiological processes whereby it is secreted.

XIII

SOME ASPECTS OF THE CHEMISTRY OF NATURAL RESINS AND LACS.

BY

D. N. Gupta, M. Sc.

THE present paper attempts to consolidate and crystallise the existing scattered knowledge of the Chemistry of natural resins and lacs of both vegetable and animal origin; such a study seems to be as imperative as it is desirable to call attention to the several gaps which exist in the Chemistry of this interesting though difficult subject, in the hope that it may stimulate further systematic and thorough investigation and thus provide the speculative physiologist with definite chemical data on which he might found his hypothesies. It must be definitely pointed out that physiologists should not rush in to theorise where chemists have not done the pioneering work and the present confusion and controversy should be a sufficient warning to them.

Although known to mankind from very early times as articles of common necessity, the real scientific study of these raw materials has been taken up only recently partly stimulated by a desire to prepare synthetic substitutes as a result of overgrowing demand due to their manifold uses in different important industries. As such it is only the past few years that bring out the systematic study of this subject. Still in some cases the constituents of these substances have not been properly recognised and the work only points to the limits of the standard analytical methods.

It is generally known that in the vegetable as well as in the animal kingdom, the performance of similar physiological functions is correlated with a similarity in chemical products. So resins and lacs, which are regarded by physiologists as secretion products in the course of normal or pathological metabolism may have some common basis of constitution.

Conifer resins, abundant and useful as they are attracted the notice of many workers. Abietic acid being the resin acid in all coniferous trees and in some others, has been the subject of much study and controversy. Though there is no dispute as to its molecular formula (C_{20} H_{30} O) the representation of its various isomers is controversial as Jonas and Locker have proved, by preparing the methylester and distilling it *in vacuo*, that Tschirch's *a*-abietic acid is a mixture of three isomers.

The general concensus of opinion points to its being a derivative of retene (decahydroretene carboxylic acid). Jonas, however, in his lecture at the German Varnish Congress, said that the reduction of abietic acid with sodium in amyl alcohol gives a hexahydro-derivative which fact precludes its possibility of being a derivative of retene. Besides, Bruhn's and Grun's suggestion of its being a condensation and oxidation product of *a*—and *b*—pinene seems to have been supported by Jonas, who isolated by reduction a hydrocarbon, diterpene (C_{20} H_{30}), as well as resin-alcohol and resin-aldeyde (C_{20} H_{32} O and C_{20} H_{30} O), intermediate products of resin acids which could be formed from terpene. Jonas further supported his formula by preparing hexahydrogenised phenanthrenes and retenes and compared their physical properties with those of hydrocarbon obtained from abietic acid and concluded that they do not belong to the same series.

The author rather tends to the view that the difference in constitutional formulae might have come possibly from the difference in the sources of the acid. In the analysis of these natural products it would be well to start with pure raw materials as obtained from plants rather than with substances obtained from some finished products.

The beginning of the year 1922 revives the dispute as to the identity of Aschan's Colophenic acid with the auto-oxidation product of abietic acid as suggested by Fahrion (*Ber., 1921, p. 1944*). But Aschan is unable to share Fahrion's view of the identity of his acid with oxyabietic acid on the ground that Fahrion's acid is not homogeneous and that his acid has been prepared in a pure state by taking advantage of the fact that in salt solution only colophenic acid can be precipitated by carbon dioxide whilst the isomeric resin-acids are not (*Ber., 1921, p. 867*).

Another important contribution of Aschan to this subject is the detection of a resin-acid in the oil of sulphite-cellulose factories (*Ber., 1921, p. 867*); this has been named pinabietic acid owing to its close resemblance to abietic acid. For some time it was held that they were identical, but the strongest argument against this view is the observation of Levy that abietic acid is converted by cold potassium permanganate into a tetra-oxy-acid, [C_{19} H_{29} (O H)$_4$ COOH], of high melting point, whereas a product prepared in the same way from pinabietic acid is a crystalline, apparently saturated mono-carboxylic acid of low melting point.

They may be isomeric. Also the hydrocarbons obtained from the acids seem to be identieal with 7 : 13 dimethyl-2-isopropyl—5 : 6 : 7 : 8 : 9 : 10 : 13 : 14—octahydro phenanthrene (*Ber., 1922, p. 2944*).

It might not be out of place now to indicate in a few lines the work on another resin which has been

studied, amber. Amber is a fossil resin which has been studied by Tschirch (*Arch. Pharm., 1915, p 253 p 290–305.*

It has been examined by the extraction method alcohol removing 30 per cent. leaving the remainder insoluble. The alcohol-soluble part contained succoxyabietic acid and succinoabietolic acid, while the alcohol insoluble portion consists of a saponifiable constituent which yields on saponification succinic acid and a succinoresinol besides an unsaponifiable constituent named by him succino-resene.

M. Henze considers that the solid constituent of storax-balsam is not a single storesinal in combination with cinnamic acid but a mixture of five or six distinct substances. The major part of the resin consists of a mixture of two isomeric acids, abietic and pimaric acid. Of course, this is perhaps the first instance of the occurence of these acids in a non-coniferous tree.

Besides those already dealt with, there is a long list of other vegetable resins finding their uses in varnish making which are classified according to their degree of hardness and solubility. Those interested in that particular branch of the subject would do well to refer to Coffigner's 'Varnishes, their Chemistry and manufacture'.

Japan-lac or more correctly Japan lacquer, though strictly speaking it can not be grouped under the subject, has been dealt with owing to its pysiological similarity with resins. This lac which finds extensive use in painting aero-planes and in lacquer works, has been the subject of systematic and continued study by M. Majima (1909—1922).

Urishiol, $C_{20}H_{30}O_2$, which is the chief constituent of Japan lac is not a homogeneous substance but a mixture of several compounds differing from one

another in the number and position of double bonds in the long normal chain, but on reduction, they give the same hydrourishiol which has been proved by Majima to be 3-pentadecylcatechol. The constitution of urishiol has been further supported by the production of veratrol-o-carboxylic acid (1 : 2 : 3) when urishiol dimethyl ether is oxidised with potassium permanganate. The dimethyl ether obtained by synthesis was proved to be identical with that prepared from natural urishiol whereby its constitution is fixed with the exception of the position of the double bonds in the side-chain. It has been synthesised from sodium dodecane and 2 : 3 dimethyl phenylpropionyl chloride followed by reduction of the condensation product.

The results of the analysis of the bromide and ozomide of the dimethyl ether, and the volume of hydrogen absorbed during reduction, point to the fact that it contains two double bonds in the molecule. In Indo-China and Formosa lac, the chief constituent is 'Laccol' which is believed by Majima to be urishiol with two methylene groups in the side chain, and 'Thitsiol'—the constituent of Burma lac is supposed to be a homologue of isohydro-urishiol with an unsaturated side chain.

Indian lac, however, differs from all others in being an animal product, the semi-solid exudation of a class of insects—coccidi, feeding on certain plants. It comprises for the most part a resin along with a dye (5-6 per cent.) and two waxes melting at 72° C. and 94° C. respectively. As lac-dye does not come under the subject, anybody interested may refer to *Annalen*, *1913*, *63*. Wax having m. p. 72° C obtained by petrol extraction according to Benedict and Gascard consists of 50 per cent. myricyl alcohol and very little ceryl alcohol, the balance consiting of the ester of these alcohols with melissic, cerotic, oleic and palmitic acids. The hard wax obtained by hot benzene

after the extraction of resin by alcohol, is an ester of the lacceroic acid ($C_{32}H_{64}O_2$) and an alcohol laccerol ($C_{32}H_{66}O$) (*Compt. rend.*, *1914*, *258-60*).

Lac resin wnich is soluble in alcohol as examined by the extraction process consists of ether-soluble (25-30 per-cent.) and ether-insoluble portions. The ether-soluble part studied by the author, contains besides a needle shaped crystalline orange colouring-matter 'Erythrolaccain', a derivative of anthraquinone, a very small quantity of an odorous principle and some fatty acids both saturated and unsaturated. The viscous property of shellac is mainly due to this ether-soluble portion. The ether-insoluble portion according to Tschirch (*Arch. Pharm.*, *237*, *35-48*) consisted of a resinotannol ester of aleuritic acid, the acid being recognised as dioxytridecylic acid, $C_{12}H_{23}(OH)_2COOH_2$. Only recently Harries and Nagel (*Chem. Umschau 1922*, *135*) proved it to be trioxy-palmitic acid by reducing it with hydriodic acid and phosphorus to palmitic acid and determining its acetyl value. The author's investigation on Mysore Lac also confirms Harries's result. From the mother-liquor left after the extraction of aleuritic acid from the saponified resin another acid dioxydicarboxylic acid of the formula $C_{13}H_{18}(OH)_2(COOH)_2$, named by Harries shellolic acid has been isolated. (*Ber.*, *1922*, *3833*) No resin-alcohol has been traced. The acid is optically active and to this acid a suggestive constitutional formula has been given by Harries (*Ber. 1922*). But in view of the work of Etard and Vale'e (*Comp. rend.*, *1905*, *1603*) on pyrogenation of lac, where they claimed to have found out a series of terpenes as main products along with some fatty acids, it is rather difficult to reconcile with all the facts unless it is known that decomposition of any of the lac constituents may result in terpene. Future work on the constitution of shellolic acid may throw some interesting light in this direction. Though at first it may appear as though

the similarities between the resin-acids are so few as scarcely to justify the writing of such a paper, the task is not altogether without compensation, the curious similarity in composition of the secretory products both vegetable and animal offering numerous suggestions for speculation and experiment; but the most interesting is the genesis of these common nuclei as a result of similar physiological processes, either in the plant or in the animal kingdom.

XIV
UTILISATION OF BY-PRODUCTS IN THE LAC INDUSTRY.

BY

M. Venugopalan, B. Sc.

IT is a recognised maxim of industrial practice that the recovery of the by-products of an industry increases its economic stability, for one is enabled to present his finished product at a cheaper rate, thus safeguarding the interests of his industry against possible competition. Lac, which has hitherto been an exclusive monopoly of India, is now fast running the risk of being supplanted by synthetic substitutes. The fluctuating and inflated prices which have often ruled the trade have encouraged the search for a substitute on the part of those who have to be at the mercy of monopolists. So far, no satisfactory synthetic substitutes have been evolved although it would be unwise to ignore such a possibility in the near future.

Efficient production, scientific methods of manufacture and economic utilisation of the by-products constitute the means by which the position of the industry may be strengthened. The present paper is devoted to a discussion of the last aspect of the question.

The by-products may roughly be divided into two classes :—

1. Those which occur during the preparation of seed lac.
2. Those which result during the manufacture of shellac, button-lac, garnet-lac etc.

To the first class belong :—

1. Residual twigs from the "Phunki lac" after the removal of incrustation.

2. Fine dust "Molamma" arising from the sieving preliminary to the washing of lac.
3. Washed liquors containing the dye, finely divided resin, insect bodies, etc.
4. Dye cakes got by precipitation of the washed liquor by calcium chloride.

To the Second class belong:—

1. 'Kiri', a refuse remaining in the bag.
2. Lac-wax.

The first by-product left in any lac factory is the accumulation of twigs got from the "Phunki lac" after the removal of lac by beating with wooden mallets or by crushing in a machine. Here we may mention that the lac adhering to the fragments of twig, especially to those of the summer season crop, cannot be completely removed by simple mechanical means and a fair percentage of resin is lost in this direction. This loss can be readily minimised by boiling the twigs in a dilute solution of soda from which the resin may be got by neutralisation with acid. The twigs after drying can be used as fuel for boilers.

Before the crushed lac is put to wash, it undergoes preliminary sieving and winnowing whereby fine particles of resin and dust are removed. The latter products form the tailings and are usually known as "Molamma". They consist of very fine grains of lac mixed with powdered wood, insect bodies, dust, wax and dye. It is very profitable to recover the fine particles of resin from them as is done at Mirzapur by a special method. It is also used at the present day as a raw material for the manufacture of second grade varnishes, and for bangle-making with necessary addition of rosin. Since the dust consists mainly of the dye and wax, attempts can be made to recover

them in a pure state as in the case of dye cakes. Instead of undertaking a laborious method of the isolation of the dye, matters can be very much simplified if the "Molamma" after removal of resin and wax is directly put on the market for the dye, since the residue is mainly of insect bodies containing the dye, which can be easily separated by shaking with water rendered slightly alkaline.

The third important by-product is the dye in the liquors resulting from washing the crushed lac. This liquor can be conveniently utilised as such, or the dye can be separated from it in the form of cakes by simple precipitation with calcium chloride. If a continuous supply of wool is at hand the most economical way of utilising the liquor is to dye the yarn directly with it. Lac dye was originally the chief product of the lac industry, and was held in high esteem for its bright red colour. Two generations ago, however, it began to yield place to vegetable and chemical substitutes, and within the space of a few years, the trade had dwindled to nothing. It is naturally fast to silk and wool, but not to cotton. It suffers from all the defects, inherent in natural dye-stuffs; it cannot be produced in a clean and workable form, and requires great care and adjustment both in quantity of the dye-stuff and proper mordants in order to produce a uniform shade. One other draw-back is that the high cost of tin and aluminium salts which are used as the principal mordants and with which only the brightest colour is obtained, withholds it from the reach of an ordinary dyer. These are the main defects of the lac dye which are answerable for its disappearance from the market, and it is obvious that if it should ever compete with synthetic dyes a means must be devised by which, the essential dyeing agent contained in it can be extracted almost pure, standardised and put on the market at a cheaper rate in a clean and handy form.

Since it is very difficult to compete with the synthetic dye-stuffs I think it is not worth while trying to isolate the pure dye-stuff. Though the dye-liquor is practically useless for this purpose, it can be utilised as a fertiliser since it contains a large supply of albuminoid nitrogen in the insect bodies. It should not be forgotten here to mention that an important defect of the dye-liquor is that if it is allowed to stand for sometime, it will begin to ferment with an offensive smell. By simply aerating the dye-liquor for a long time there is every possibility of rendering it inoffensive and at the same time of developing a sludge which would disintegrate and oxidise the insect bodies to a useful fertiliser leaving the dye in a fairly pure state.

The dye-cakes got by precipitation with calcium chloride consist mainly of the dye, partly of the insect bodies, some resin and a small percentage of wax. Methods that have been usually recommended for the utilisation of these cakes are:—

1. as a fertilising material since it contains a rich supply of nitrogen derived from insect bodies.
2. as fuel for boilers and
3. for the preparation of low grade varnishes; wax and pure dye.

Though lac-dye has lost all its chances of being used as a dyeing material, there is every possibility of converting it into a beautiful lake which might find a fair market. We all know that Cochineal at the present time is consumed largely for the preparation of the lake "Cochineal carmine", which is used to a great extent in water colours, paintings, and confectionery. Since lac-dye closely resembles Cochineal, it may also be used to give a lake with tin or aluminium, yielding a product very much akin to "Cochineal

Carmine " and which could be used for the same purposes.

In all lac factories, grain-lac forms the starting raw material for the manufacture of shellac and other grades of resin. During the process of manufacture two important by-products arise and could be profitably utilised for many purposes. It is unnecessary to discuss in detail the different methods of manufacture of shellac. It will suffice to mention that it is effected by two methods, either by blending and fusion or by solvent extraction. The former process is used largely at the present day, though the latter possesses this distinct advantage, that shellac completely free from wax can be prepared rapidly and in large quantities. In the second method also, there is the difficulty of removing the last traces of alcohol without affecting the solubility of shellac. Whichever process of manufacture is used, the by-products are more or less of the same character.

During the manufacture of shellac by the blending process, a large quantity of refuse or " Kiri " is accumulated in the portion of the bag exposed to the fire after the shellac has been squeezed out. It is a black sticky mass, consisting of animal remains, woody material with a large proportion of lac resin and a considerable quantity of lac-wax. It has been utilised for preparing garnet-lac by the solvent method, cheap grades of shellac and also for making toys and bangles.

The last important by-product in the shellac industry is lac-wax which has not been worked up as a commercial product, because cheap and suitable methods for recovering the wax from "Kiri" "Molamma" etc., have not been perfected. At present it is only obtained by precipitation during the manufacture of bleached shellac. On a small scale and in a pure form it can be extracted from lac with low-boiling

petrol. When pure, it is white in colour, harder than bees-wax and can be used to replace bees-wax in industries.

The important uses of by-products in the lac industry are sufficiently indicated above, and are not only of great economic importance to India, but would also serve to consolidate this Indian industry in spite of a possible synthetic substitute being discovered in the future.

XV

ADULTERATION OF SHELLAC

BY

M. Rangaswami, B. A.

INTRODUCTION.

ADULTERATION is the violation of the purity of a product by the admixture of foreign substances. If one has this rigid view of adulteration in mind, one will be greatly surprised to find the large number and varied nature of the substances that are found in shellac and its products: common rosin or colophony, kauri, copal and sandarach, orpiment etc. On analysis of some specimens of shellac, they were even found to give tests for sulphuric acid, phenols and aldehydes. The presumption would naturally be that the shellac was mixed with the condensation products of phenols and aldehydes. In view of the expense, however, it is very difficult to say whether products, like these and the metallic resinates, ester gums and glycerine—rosin compounds, which are also reported to have been used for adulteration, are really used for that purpose or are only found there by accident.

The practice of adulterating shellac with rosin and orpiment, nevertheless, dates back to the earliest days of the lac industry in India and has been still prevalent in spite of the bitter complaints of the buyers on the foreign market. The abnormal difference in the prices of shellac and rosin is a sufficient temptation for the local manufacturers of lac to introduce more quantities of the latter into their produce than can be easily overlooked and samples of shellac are put on the market which reveal an adulteration with rosin as high as 20 to 50 per cent., the T. N. and Ralli brands being most adulterated. This extensive adulteration is

largely assisted by the fact that, for a long time past, no reliable methods of detection and estimation of the adulterants were available and even at the present time many of the methods of analysis are far from being standard and accurate to a high degree. In the absence of a specification, therefore, as regards the purity or permissible adulteration of the product and purchasing it on the results of chemical analysis, the composition of the product varies from year to year and even from seller to seller. The consequence would inevitably be that the Indian Shellac, by losing credit, would give room for the manufacture of more and more of the synthetic product which is "inferior in hardness and elasticity" to the natural shellac, but nevertheless "superior in polish and lustre".

Adulteration with orpiment is even more scandalous than adulteration with colophony. In spite of the protests of the users of shellac or shellac products in some industries, the fusion of shellac with orpiment continues on the pretext that the latter assists in improving the colour of the product. The arsenic content of some specimens goes up as high as 4—2080 parts in a million and it is certainly bad that such a product should be used by confectioners in glazing their goods or in brewery for coating the fermentation casks.

DETECTION AND ESTIMATION OF THE ADULTERANTS.

As has already been observed, the analysis of shellac has not been systematised in spite of the great interest the subject has aroused in India as well as abroad. Standard factors of chemical analysis have to be fixed and the certainty and accuracy of the methods of obtaining them to be improved.

The colour of different grades of shellac was for a long time a rough and ready method of judging the purity of the substance, but it is found to be unreliable and apt to lead to erroneous conclusions.

Storch and Morawski have developed a method in which the sample is dissolved in acetic anhydride and a drop of concentrated sulphuric acid is added to the resulting solution; a brown colour is said to develop if rosin is present. But the test is uncertain when the sample contains under 10 per cent. rosin (*Journ. Soc. Chem. Ind. 1905, p. 15*). Langmuir's modification of the reaction (Ibid) claims to work with even 2 to 3 per cent. of rosin. A calorimetric test with Wij's solution for rosin in shellac varnishes is to add 5 C.C. of the solution and 5 C.C. of glacial acetic acid to 5 C.C. of the varnish, when the presence of rosin is indicated by a reddish brown coloration (Ibid). Halphen's colour reaction is claimed to be not only able to detect the presence of rosin as low as 0·2 to 0·3 per cent. but also to estimate quantitatively the amount present in the sample (*Journ. Soc. Chem. Ind. 1909, p. 151; 8th Int. Congr. Appl. Chem. Sec. V/e, Orig. Comm.; original paper Ann. Chim. Anal. 1909, pp. 14—17.*). The presence of other resins can also be detected by the colour changes observed during the test. The method, however, fails when more than a trace of ether, alcohol or water is present (*J. Ind. Eng. Chem. 1911, p. 86*, and *Journ. Soc. Chem. Ind. 1911, p. 273*) Ingles's (*Journ. Soc. Chem. Ind. XXXI, 1912, p. 273*) and Twitchell's methods and Parry's modification of the latter (*Chemist and Druggist, 1903, January 31.*) aim at the same object. Another novel and interesting method of detecting some of the resins is that developed by Wolff (*Farb. Ztg. 1916, pp. 21, 1198—200, 1222—3*) in which advantage is taken of the different so-called precipitation points of different resins in alcoholic solution.

It has now been generally agreed upon that an estimation of the iodine-absorption value of shellac gives an accurate idea of the percentage of rosin present, the best method of doing it, however, is still a controversial point. This particular constant has been

chosen for the purpose on account of the big difference in the figures for the two resins. Hübl's method consists in leaving a weighed quantity of shellac dissolved in alcohol in contact with an excess of alcoholic solution of iodine and mercuric chloride for 24 hours and titrating back the excess of iodine with standard thiosulphate. Wij's method which is an attempted improvement of the older Hübl's, employs instead an acetic acid solution of iodine into which chlorine has been passed until the titre is doubled and ½ to 1 hour is claimed to be sufficient for the absorption of iodine by the unsaturated compounds in the resins. W. B. Parker has published a paper (*J. Oil and Colour Chem. Assoc. Vol. V. 1922.*) in which he expresses his opinion that Hübl's method gives the real iodine absorption value and contends that the other process always gives a higher figure indicating thereby that not only an addition of iodine takes place in the latter process but also a partial or complete substitution with iodine or iodine-chlorine compounds. This view is strengthened by Anton Pecararu (*J. Oil and Colour Chem. Assoc. Vol. V. 1923*). The other methods, viz., Hanu's and Winckler's are very little in use in the analysis of resins.

But, whatever the method employed may be, from a determination of the iodine value the amount of rosin present in a sample of shellac could be estimated. The percentage of rosin could be calculated by the formula $x=\frac{100\,[M-S]}{[R-S]}$ where S, R and M represent the iodine values of shellac, rosin and the sample respectively and X, the percentage of rosin in the sample. It is however more difficult to estimate the rosin in bleached shellac, since the former materially loses its power of absorbing iodine after going through the process of bleaching.

Next in importance comes orpiment, which for a long time successfully avoided detection on account of the difficulty of applying tests for its presence. A

microscopic method has lately been developed which professes to find out even small percentages of this adulterant. Other methods of detecting and estimating arsenic are given by Langmuir and White *(Journ. Soc. Chem. Ind., 1911, p. 786).*

Sandarach could also be tested for by a method other than the general one for the resins *(Chem. Abs. XI p. 1816).*

GENERAL OBSERVATIONS.

It is worth considering now the question of minimising, if not suppressing totally, the tendency of adulterating shellac. The practice may be reduced to a certain extent by placing the whole lac industry on a better basis than it is at present. The methods of propogation and collection of lac are still far from being satisfactory. Enough attention has not been devoted to the problem of ascertaining the conditions determining the best crop and the quality as well as the quantity is left more or less to chance. It is only very recently that experiments are being conducted on the subject of the nutrition of the lac insect and its host-plant, two most important factors in the cultivation of lac. It may be mentioned here that some very useful work is being done in this direction in the Bio-chemical laboratories of the Indian Institute of Science. The manufacture of lac has also to be conducted on more economic lines than at present, not neglecting the question of the utilisation of all the waste products in the industry. Placing the whole industry thus on a more scientific and economic basis no doubt conduces not only to increase the output but also to bring down, as a consequence, the cost of production. Shellac could then be put cheaper on the market and the practice of adulterating it with rosin would certainly be diminished. A purer and more uniform product would then be available.

Another way of abolishing this practice is the specification of standards for shellac, but this entails the systematisation of the methods of analysis of the resins so as to give concordant results irrespective of the experimenter or the method employed. An attempt has been made by W. B. Parker, *(J. Oil and Colour Chem. Assoc., Vol. V. 1922)* in this direction, who, after a consideration of the results of complete analysis of shellac, suggests the following factors as suitable for specification :—

A. CHEMICAL.

1. Moisture, per cent.
2. Mineral matters, (ash) per cent.
3. Total non-resinous matters, per cent.
4. Iodine-absorption value, per cent.
5. Acid value (direct and back titration).
6. Saponification value (hot).
7. Rational Composition, per cent.

B. PHYSICAL.

Gross Solubility, per cent.

C. PHYSICAL CHARACTERISTICS.

Colour, size and habit.

The specification of the range of iodine value restricts the extent of adulteration with rosin. For draft specifications of some varieties of shellac, reference can be made to the original. If a specification like the above, is imposed on all shellacs put on the market, one is always sure of the quality of the product one is purchasing.

It is not, however, necessary to demand that only rosin-free shellac or shellacs containing very low percentages of rosin should be marketted. If an industry, in which shellac or a shellac product is employed, permits a more extensive adulteration then it is purposeless to select the purer varieties of shellac, probably paying a somewhat higher price. It is

worthwhile, therefore, for one who is interested in any large industry in which shellac or any product prepared from shellac is used to find out not only what composition of shellac will answer his purpose but also what is best. A specification of his requirements would therefore not only materially assist placing on the market a product which is particularly suited to an industry, but would couduce to make more shellac available for purposes demanding the purer varieties.

XVI
TROPICAL VEGETATION—THE FUTURE SOURCE OF LIQUID FUEL.

BY

Bholanath Banerjee, M. Sc.

THE modern age is the age of prime movers and machineries. We are at the end of the coal stage which was considered to be the best source for power and energy. Super-speed and high efficiency have put steam engines into the background and liquid fuel and oil-engines are the order of the day. Modern developments require automotion rather than steam traction, or aviation. This requires that the demands of progress should be supplied. The mining of crude oil has increased two-fold in the last ten years, but the use of autos has gone up nearly ten-fold. Future commercial development of aviation would mean a still further increased demand for the best liquid fuel. This growing demand has necessitated a great activity in the production of liquid fuel and is met to a limited extent by cracking the heavier fractions, increasing the range of distillation cut, recovering light oil from natural gas, etc. In this way the output has been increased from twenty to twenty-eight per cent. of the crude output. This enormous production has assumed such huge proportions that already reserves of oil are being depleted and exhaustion of the resources in the near future is threatened. Petrologists and geologists surveying for further supplies of unmined oil are publishing alarming reports and foretelling a famine in about twenty-five years. This means that other sources must be found and utilised to the fullest extent.

The use of *shale, peat*, and the like do not appear promising sources so far. Two other liquids, benzene

and alcohol are used nowadays to a small extent as adjunct to gasoline. They are promising substitutes and may replace it in future.

Benzene is a good liquid fuel as such or in conjunction with alcohol or petrol. The present production, however, is only 3 per cent. of the liquid fuel demand. Besides, it has its use in other industries, notably dyestuffs. Even if we can use all our coal to increase the benzene supply, this will provide only five per cent. of the total petrol output.

The next choice, *alcohol*, appears to meet this problem fully. It is obtained from a source that can be renewed from year to year without exhaustion of the source or impoverishment of the stock like petrol or benzene which Nature has taken a long period of time to build up and store. In fact it is one of the direct routes we command for converting energy from its source, the Sun, through vegetation to alcohol to be used in engines.

In the *tropics* the demand for liquid fuel is small but it may not be so in future. There are no good deposits of petroleum to count upon, those of Burma and Persia which supply only seven per cent. of the total world output has many outside consumers to meet. They cannot be expected to last for more than forty years. Whether in times of peace or war, we should be self-supporting. We have no reliable or sufficient source for mineral oils. But modern state of competition requires that we should at least be in a position to meet our demands. Germany on account of its foresight twenty years ago introduced alcohol in arts and industries as also for power purposes. Nature has been bountiful in another way. Land is more generally available, labour is cheap and plentiful, with abundance of rain and sunshine. Climate is just what is suitable for the required vegetable. There

are again vast quantities of annual vegetation of little or no commercial value which if properly utilised may serve as a cheap possible raw-material for the production of alcohol.

Alcohol meets the demand of a liquid fuel from stand-points of cost, boiling point, calorific value and general efficiency. It has been tested ever since 1896 on exhaustive lines as a motor fuel. It has certain drawbacks, still it has been found to meet all the requirements of a suitable liquid fuel and indeed it has certain advantages over petrol. Since the beginning of this century, over seventy five per cent. of the production has been used in industries and for power in Germany who has to depend on outside countries for petrol.

At present the only commercial methods of proved economic value for producing alcohol are fermentation processes which utilise sugar obtained directly from the plant e. g., molasses or from starch grains of all kinds. *Molasses*, one of the by-products of sugar manufacture, is one of the best materials for power alcohol. Unfortunately, if all the molasses are used for this purpose, we can get only six per cent. of our demand. In India and similar countries, it has use in tobacco manufacture and potable spirits, leaving very little surplus.

The starches from the cereals, if available, can at once meet nearly the whole fuel problem. But is it available, though sufficient quantity is grown year after year? The most important fact that cereals are our principal food-stuffs stands against the use of this as a raw material. With constant famine menace and shortage of food supply, it is out of question to use cereals for solving this problem.

The less important cereals like ***maize, millet*** and ***sorghum*** are worth consideration. As foodstuffs they are not so valuable as rice or wheat and give a

heavier crop per acre. If the stalks be used as source of paper pulp or for the production of alcohol, the crop is doubly useful and may be expected to meet the demand to a limited extent.

In India, *Mohua flowers* appear as immediate sources to a limited extent. They are used to a certain extent as cattle food and for the manufacture of potable spirits, but are not fully utilised. Dried flowers yield 30 per cent. of their weight of alcohol. If grown on a plantation scale of 15 to 20 trees per acre they will yield a higher quantity of alcohol than the best of cereals and at a lower cost. The trees are very resistent and grow to a big size, giving useful and very hard wood. They grow in many dry places where few other trees can be grown and thus many waste and dry regions can be utilised. The chief drawback with this source of alcohol is the uncertainity of the crop and the long time that must elapse before the trees come into bearing; also the cost of collecting and storing the flowers. On the other hand, during the period of growth other crops can be grown and the cost of cultivation will be nil after maturity. Mohua seeds are an important article of commerce giving a valuable edible fat and oil cake. It is estimated that dry regions of Gujarat, Central Province and Hyderabad can supply ten million gallons of liquid fuel annually.

Cellulose offers itfelf as a good material for the supply of motor fuel. It is cheap, readily available, and its supply is renewable. By the return to the soil of the refuse material from the production of alcohol, the soil would lose none of its constituents to keep up its fertility. But a number of problems have to be solved before cellulose material can be used to serve as the raw material for future motive power.

The first is the commercial development of a process that will give workable yield of alcohol from cellulose. As early as 1826, Braconnet demonstrated

that wood cellulose on hydrolysis gives sugars, and alcohol on subsequent fermentation. Since then a good deal of work has been done in this line and experimental plants worked to test commercial possibility. Cellulose can be converted quantitatively into sugars by means of a large quantity of strong acids, but the cost of materials and the difficulty of their recovery militate against this process. However, during the past five years commercial plants have been working in France and Germany, where they use this process of hydrolysis and are said to have overcome the difficulty of recovering the acid and using it again. They are said to obtain from 40 to 50 gallons per ton. Cellulose can also be hydrolysed to sugars on being subjected to the action of dilute acids at high temperature and pressure. *Simonsen, Clässen, Kressman, Tomlinson,* make use of this process. A few plants have been working in America since 1914 producing over a million gallons per annum. The drawback of this process lies in the fact that though nearly thirty per cent. of reducing substances are obtained on hydrolysis only six to eight per cent. of alcohol is obtained on fermentation. This is a low figure being only 15 to 25 gallons per ton. Wood substances which contain over fifty per cent. cellulose should yield at least sixty to sixty-five gallons per ton. But the fact of the quantitative yield in the strong acid process, and the cheapness of the dilute acid digestion process, holds the hope that there is a commercial possibility in this line and justifies further research being done to develop a commercial process. If this result is achieved then we may say on the present basis of cost of raw material and manufacture that the problem of liquid fuel is solved.

The next and equally important question is the supply of raw material.

Forests:—The annual growth of wood in the forests of United States of America is estimated to be six billion cubic feet. The possible growth if properly

restocked after cutting and protected is estimated to twenty-seven billion cubic feet. But all this will not be and cannot be available for liquid fuel. Lumber and other industries require roughly 35 per cent., wood fuel 40 per cent., other products 16 per cent., and 6 per cent. is destroyed by fires, insects, fungi, and other pests. 15 per cent., is wasted and lost in the woods and mills. This waste and that from fires and insects say 20 per cent. or nearly six billion cubic feet can be available for liquid fuel supply without encroachment on any other demand. Further more with intensive forest crop management, particularly the removal of small wood and thinnings should not amount to less than ten per cent. of the total growth.

How much can this total yield as liquid fuel? Calculating on the present worked yield of 20 gallons per ton this will yield 2·4 billion gallons or 33 per cent. of the total output of petrol. At present this costs 25 cents per gallon. The Standard Alcohol Company of the United States of America state that under best and large scale conditions the cost of production can be reduced to 10 cents per gallon. Here science must come to the rescue by providing better and efficient method. Such a process if developed will be a double blessing; it will supply liquid fuel from a waste as also make intensive afforestation possible by removing unsaleable waste which gives rise to fires as also serves as the breeding ground for insects, pests, and the like. Secondly, by removing small trees in thinnings intensive forestry becomes possible.

It might be sometime to see such a process actually worked, nevertheless, it is important to have the largest and best supplies of wood at the time, that forest areas without delay be protected, scientifically cut, and restocked.

One difficulty with wood waste is that it is enormous in bulk, and the cost of assemblage and transport is high.

Then we have other sources like straw, hay, grass, corn stalks and cobs, megasse and bamboos to reckon with since they are assembled before hand for their primary product.

Hay and Straw are available in large and sufficient quantity every year. It has other use as cattle food and as farm-yard manure, still they are improperly used and wasted. In many parts, in the deltaic regions of Bengal and Burma, where rice is grown far in excess of local demand, and where there are plenty of rivers for transport facilities, millions of tons of these are available and should a process be worked to saccharify and ferment the cellulose, they may serve as modest units for supplying a fair quantity of alcohol.

Corn cobs, corn stalks and *megasse* are well worth consideration as they are more or less improperly used or wasted by-products of agricultural industry. A second mill for preparing pulp or alcohol from the cellulose may be a valuable adjunct to the primary industry.

The bamboos and the perennial grasses present the best supplies so far. They are very easy to develop and require little or no attention during growth. Being resistent they are little affected from the attacks of insects, fungus, or the cattle. Whitly calculates that acre per acre in the tropics, with plenty of rain and sunshine the growth of vegetation is five times quicker than it is on any other part of the globe. He estimates that a plot of land measuring 600 miles by 150 miles would be sufficient in the tropics to yield as bamboos or tall grasses, enough of cellulose to produce the annual requirement of liquid fuel. In India the hills of Assam, the terais of the Himalayas and the forests of Burma with plenty of hill streams and rivulets for transport offer as the best sites to work at this problem.

But the crux of the problem is, can any process be possibly devised. There is a way that promises better. No one who has compared the processes of nature with those of the most expert chemist or industrialist in the laboratory or factory could help to have been struck with the beauty and efficiency of the former and the comparative inefficiency and clumsiness of the latter. It is the use of the micro-organisms or their enzymes. The changes brought about by these are comparatively speaking the cheapest to work upon, on account of the efficient manner in which they operate. Organism that can saccharify or ferment cellulose have an important bearing on this subject.

So far no organisms are known that would convert cellulose into sugar or alcohol. But others are known which eat away cellulose and thus remove them from the surface of the earth. They produce carbon dioxide marsh gas, volatile acids, and the like. What is required is that we should have some organism or symbiosis of organisms like that of horse dung, manure heap, pond mud, or the septic tank mud that would contain a cellulase and an amylase to break down and saccharify. The selection and isolation of the organism is absolutely necessary.

If such an organism and process is devised then it is possible to imagine a picture of our future supply of liquid fuel as resulting from a direct conversion of sun's rays, through the medium of vegetation, the celluose of which is first converted by a bio-chemical process into cellubiose, glucose, and finally into alcohol.

It will be no small laurel on the crown of one or the band of men who can in the near future, announce the completion of the first commercial unit in which cellulose material could by the action of sturdy micro-organisms be converted into the final product, alcohol. For the present the scheme outlined above may be a scientific dream, but wilder fantasies have been proved

capable of realisation. Patient and intensive research is the only key note that can help towards such a marvellous realisation of this important and fascinating problem.

Alcohol and prohibition :—The prohibition act defines alcohol along with whisky and beer as an intoxicating liquor. Every chemist knows that 95 per cent. ethyl alcohol cannot be used as a beverage, but the great ease with which it can be diluted and consumed as a beverage has caused it to be classified as an intoxicating liquor. But high proof alcohol when rendered unfit for drink can be had tax free when used for other purposes. This duel phase of the law has given rise to much trouble.

A barrel of pure alcohol, tax paid, costs Rs. 1000 and when converted into bootleg whisky sells for the high figure of Rs. 15,000. This tremendous profit is the lure that attracts the thief or the criminal. But when properly denatured such a conversion is impossible.

Chemists and industrialists however want alcohol on an absolutely non-beverage basis, for it is as important and necessary as steel and mortar are for the structural engineer. No less than two-thirds of the present production is denatured and used in arts and industries. Again in alcohol industry we cannot neglect the question of national defence. A chemist forsees the future wars to be of gases, aeroplanes and high explosives. Much has been said of a self-contained dye industry with its useful peace time production and conversion into defensive and offensive weapons in war time. Alcohol industry may be well placed in the same position.

But the law must be enforced, and I believe it can be done in a way, that will conserve the good and eliminate the bad. This requires that thousands of specific products should have to be prepared with tax free alchohol and hundreds of formulas provided for specially

denaturing the alcohol. This opens up an immense field of constructive research involving problems of chemistry in its different branches and pharmacy. Let us not, as Chemists, confess our inability to solve this aspect of the alcohol problem.

XVII

THE MANUFACTURE OF ACETONE IN INDIA BY FEREMENTATION PROCESS.

By Anant, G. Gokhale, M. A., M. Sc., A.I.I. Sc.

THE story of the Acetone manufacture in India has several interesting features. It is a story of the hard experimental work and the great uphill task in designing a well-equipped factory on the part of the early workers and carrying out most of the plans and successfully working the plant on the part of their successors. On the other hand a great deal of misunderstanding was entertained by the public at large regarding the whole project, even when the place was working satisfactorily.

In England several Distilleries were turned into Acetone Factories during the war, while the fate of the Acetone Factory in India was quite the reverse. It was started as an Acetone Factory and after working for two years was disposed of for turning it into a Distillery.

During the War Acetone attracted serious attention on the part of the military authorities. It is the solvent used in gelatinising the various ingredients of Cordite. In pre-war days the chief source of this chemical was the continent of Europe and the United States of America where it was produced from wood distillation. But due to the exigencies of war these sources became precarious and it was considered necessary to make India self dependent for its requirements of Acetone for the cordite manufacture, which is carried on in the Nilgiris. In 1914-15 considerable work was done on the Weizman fermentation process for the manufacture of this chemical in the United Kingdom and a large plant was set up at Royal Naval Cordite Factory, Holton

Heath, which was followed by others at Kings Lynn and other places.

Dr. Gilbert J. Fowler, who came out to India as Professor of Applied Chemistry in the Indian Institute of Science, Bangalore, early in 1916 was charged by Government with the task of working out a scheme for the establishment of an Acetone Factory in India.

Major E. Moore Mumford the first Superintendent of the Acetone Factory was mainly responsible for the design and the early spade work of the construction of the Factory.

Dr. Fowler, Mr. Y. D. Wad, and the present author did all the preliminary experimental work in the Applied Chemistry Laboratories of the Indian Institute of Science, Bangalore. Here we had fitted up a small aluminium vat of 800 gallons capacity with a bub vessel of 25 gallons. There were two stills belonging to Applied Chemistry Department. The distillation plant available in the applied Chemistry Department was used, viz :—

i. 100 gallons prelimenary distillation still.

ii. 10 gallons still and rectifying column.

Fermentations were carried out with this plant on a semi-factory scale.

The original idea was to use Mohua flowers as raw material following on the fact that wort was successfully fermented in England. Consequently Nasik was fixed upon as a central place for this raw material, being near Hyderabad (Deccan), Gujrat and the Central Provinces which are the three large mohua growing tracts. But somehow or other this raw material did not prove very encouraging. There seems to be something present in the mohua which inhibits the action of the Acetone organism. This problem is engaging the attention of the author. Search was made for another suitable raw material.

In England maize was being used for fermentation. Jawar is a grain akin to maize and was found to be as cheap as mohua at that time. Experiments with this raw material were very successful. It was therefore decided to use this raw material in the factory, Nasik being a central place for this raw material also.

Experiments were made at Bangalore regarding the disposal of spent wash by filter pressing and dealing with the effluent by the activated sludge process. The pressed cake of the residual carbohydrates was found to be a suitable cattle-food and the activated sludge experiments gave certain interesting results. However a great deal of work remained still to be done on this subject. Experiments were also carried out regarding the limits of inflammability of Acetone vapour and air mixtures. Specific gravities of butyl alcohol and water were determined and other cognate subjects were tackled as far as possible.

The work done at Bangalore has already been published in the Journal of the Indian Institute of Science, vide Vol. 4 part I, pp. 1 to 15, and part II, pp. 17 to 25.

It is therefore proposed in this article to give a general description and a short summary of the work done at the Government Acetone Factory, Nasik, and the details of the process followed.

The Acetone Factory at Nasik which was under Government of India comprised an area of about 130 acres with 25 acres for sewage disposal. The buildings were commenced in the middle of 1917 and the Factory was ready for starting manufacturing operations in September 1919. The Factory proper consisted of:—

1. Vat House containing ten closed cast iron vats each of 25,000 gallons capacity with stirring gear, heating and cooling

coils; and eight aluminium seed pots each of 800 gallons capacity with steam and water connections and stirring gear.

2. Cooker House with a battery of four steel cookers, stirring gear, water and steam connections, pressure gauges, thermometers etc. and four wooden hoppers.

3. Grain Mill of Robinson make with four pairs of rollers with cleaning devices and automatic weighing machine. The flour was fed into hoppers by a screw conveyer.

4. Refrigerator House containing three sets of Ammonia Compressors to cool the water for condensers of the stills.

5. Still House containing two Blair Campbell and MacLeans triple column continuous stills and one pot still for rectifying.

6. Boiler House containing a battery of seven loco-type boilers.

7. Power House of three units of steam engines and generators each of 250 H. P. Alternating current being producted at 650 volts.

8. Rat Proof Grain Stores.

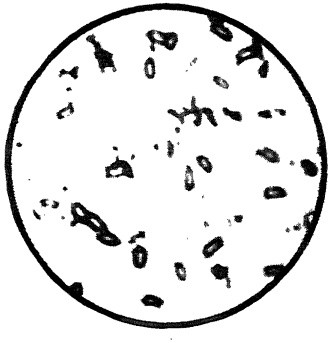
9. Underground steel tanks for storing Acetone and Butyl Alcohol.

10. Centrifugal Pumps for distributing water.

11. Water Filtration Plant consisting of two Jewell Filters, settling tanks.

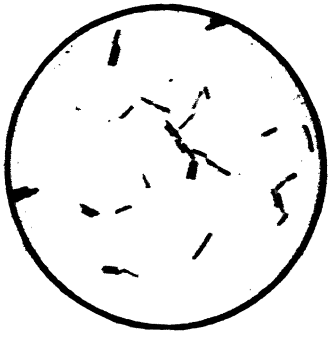
12. Stores.

B. Y. ORGANISMS

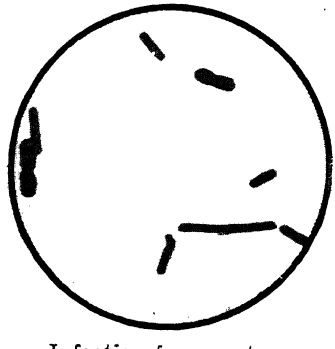


Spores

Groups



Scattered field



Infection from water

13. Electrically driven centrifugal pump at the Darna River about 2 miles away for pumping water to the filters.

14. Two Sumps to collect spent wash and sewage, to be pumped on to sewage farm.

15. Officer's Bungalows, and quarters for staff and coolies, Offices and Laboratory.

The Factory is situated near Nasik Road Station with a railway siding in the factory.

The boilers generated steam for heating purposes and the stills and for driving the engines for power generation, which was used for driving pumps and stirring gear, Ammonia Compressors and Grain Mill and for lighting purposes.

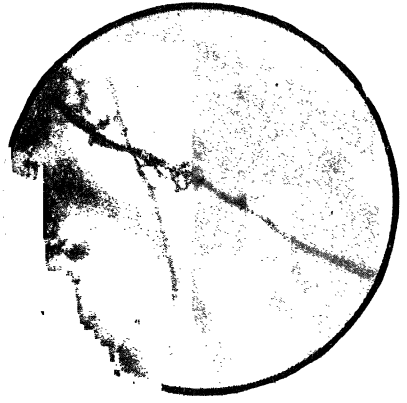
The micro organism used in the process belong to the Amlyo-bacter group of the long rod type, which is stained readily by Carbol Fuchsin, but only slightly by methylene blue. This organism produces Acetone and butyl alcohol from cereals containing starch in proportion of 1 : 2.

It forms true endospores, the spore being oval in shape the envelope only taking stain while the central portion remains transparent. Spore formation takes place at an early stage when conditions are not favourable, and a vigorous culture does not sporulate before 20–24 hours. In the Laboratory, a culture is made in a five per cent. mash. Special care had to be taken in making pure cultures for the Factory. Every week fresh culture was started from a fresh spore tube. The culture tubes were about 12 inches long by one inch in diameter. The mash for these was prepared as follows :—

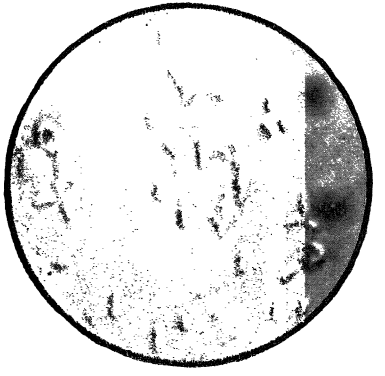
A measured quantity of water was warmed in a pan to 50°C., flour was then added gently stirring all the time to prevent formation of lumps. The liquid

was then gently boiled for ten minutes, transferred to a flask and sterilised in the auto-clave at ten pounds pressure for one hour. The tubes previously sterilised were then filled about half full with this mash, plugged and sterilised at fifteen pounds for six hours, and when cooled were kept in the incubator at least for forty-eight hours before using. The spore tube was pasteurised at 70°C for half an hour before inoculating into the mash tube. The inoculation was done with a long sterilised pipette, inoculant being introduced at the bottom. In twenty-four hours the culture used to start. It was carried over in a fresh tube and a third or fourth generation was used for the pails. The organisms from the tube at twenty-four hours are regular and thin shaped with characteristic groups. But the organisms from the vats, using coodie rice, were rather long and thick during the first eight or ten hours, almost appearing as an infection, but soon afterwards they assumed the usual form.

When the tube culture is normal, one of the tubes was inoculated into a two gallon aluminium pail. The pail was designed by Mr. A. Appleyard the last Superintendent of the Acetone Factory, and now of the Bristol University, Fruit and Vegetable Research Station. As will be seen from the figure, (p. 107) the pail is spherical in shape fitted with a thermometer pocket and a brass screw cap with a tap for screwing a pressure gauge or a nipple with an air filter. When sterilising the nipple D was put on with a plug of cotton wool and tied with a grease proof paper. Tap C and valve A were open. Five per cent. rice mash was prepared in a glass enamelled pail free from lumps. The pails were charged with two gallons each by removing the cap B. The cap was then tightly screwed and the pail sterilised in the autoclave for six hours at fifteen pounds. It was then allowed to cool and kept in the incubator. It was re-sterilised before inoculation

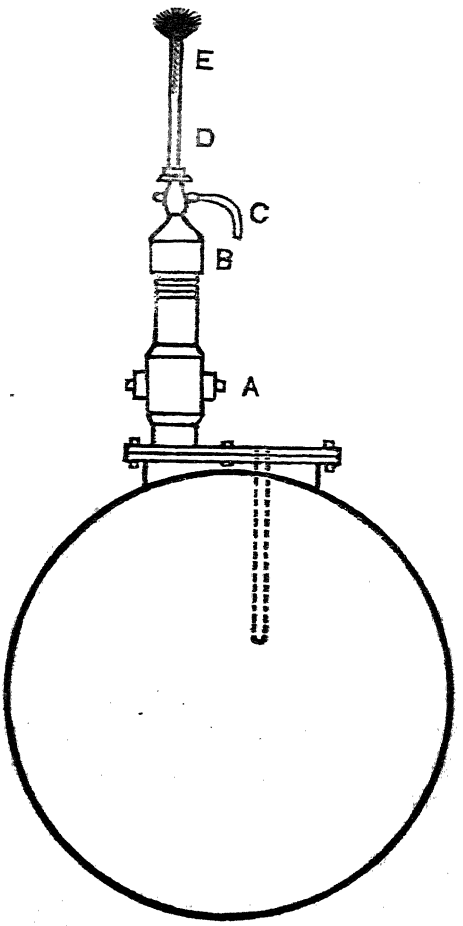


Cotton hair showing the growth of Fungi on it, on exposure to a moist warm cond'n. It is also noticable how the 'mycelium' of the Fungi actually penetrate through the canal of the hair.



Bacteria isolated from the Mildewed Fabrics.
X 1200

for two hours at fifteen pounds and an air filter E with a sterile rubber tube put on. The inoculation was done by first spraying the space surrounding with lysol unscrewing cap B slightly opening for inserting the mouth of the culture tube, the contents of which were well mixed by gently stirring. The plug was first burnt and removed and the content quickly poured into the pail, the cap B screwed on and the pail shaken and kept in the incubator. When about eighteen hours old the acidity was determined and the organisms seen under microscope. If found fit for inoculating seed pots the nipple D was removed fisrt closing the tap C. A pressure gauge sprayed with lysol was screwed on. The pressure soon rose to fifteen pound at which it was maintained for inoculating the seed pots. At the time of inoculating the seed pot the tap C was closed. Pressure gauge removed, a sterile nipple screwed on and connected with the nipple at the bottom of the seed pot with a sterile rubber tube, the tap was opened and the gas pressure in the pail forced the inoculant into the pot.



APPLEYARD PATTERN PAIL CAPACITY 2 GALLONS

The seed pot of 800 gallons capacity was fitted with cooling coils and live stem jets. The pot was half filled with water, 400 pounds of flour introduced gradually into the water which was previously warmed to 50°C. the stirrer being kept on. The manhole cover was then tightened and the pot allowed to boil. When the steam issued freely through the air filter and gas valves these two were closed and pressure allowed to rise to twenty pounds at which it was maintained for six hours. Then the pot was cooled by turning cold water into the coil, the air filter and gas valves were kept slightly open to be sterilised by the escaping steam. When the pressure dropped to five pounds air filter valve and gas valve were closed and the filter screwed on. The gas valve was covered with cotton wool dipped in phenyl. The steam pressure was then brought down to zero by opening the safety valve and the air filter valve was then immediately opened. The cooling was continued till the temperature fell to 37°C. at which the pot was inoculated.

The fermenting tanks were of cast iron plates which were bolted together and secured by rust joints. These vats could not stand pressure hence the mash for these had to be prepared in cookers. The raw material which was found most suitable and cheap at the time were low grade coodie rice from Burma. Comparative fermentations of various samples were done in the Laboratory and the yields determined. From the results the particular kind required was ordered out.

3,000 pounds of flour were fed into each hopper. The cookers were half filled with warm water from a cooling vat. The stirrers were put on, steam opened and, the flour introduced into each cooker gradually. When this operation was finished the manhole covers were bolted and the cookers were brought to boil and the steam freely allowed to escape through the

bye pass valve for five minutes when it was closed and the pressure allowed to rise to twenty pounds. The cookers were maintained at this pressure for seven hours. Top steam put on to raise the pressure to thirty pounds at which the contents of the cookers were blown into vats.

Previous to this blowing over a vat was first thoroughly cleaned, leaky joints in the cooling and steam coils repaired, and rinsed with water. About 8,000 gallons of warm water from another cooling vat were put into this vat. The water was then boiled by introducing steam into this coil for six hours. Steam was allowed to issue freely through the air filter chimney. A sample of water from the out-let valve was examined under microscope and if found free from any other bacteria or cocci except the spores of B. Y. it was declared fit for blowing the mash over. Steam was kept on the mash line all the twenty-four hours. Before blowing over water was turned on cooling coils and the air filter put on. The top steam in the manhole was opened to prevent any impure air from being sucked through the manhole. All the four cookers were blown over one after another. The manhole cover was then closed and cooling continued till temperature reached 37°C.

The chief thing in the cooker is the efficient stirring which should prevent the forming of cakes, secondly, even distribution of steam. Care also must be taken that the air valve is closed after steam issues freely to prevent any false pressure being generated.

Generally two seed pots (i. e. 1500 gallons of inoculant) about twenty to twenty-four hours old were used for a vat of 20,000, gallons. We had, however, successfully fermented 12,000 gallons of three per cent. mash in the vat with an inoculant of six gallons (three pails only). The inoculant was introduced from

top and well stirred in. A normal fermentation would finish within thirty-six hours.

The initial acidity of coodie rice mash varied from 6 c. c. to 2 c. c. of $N/_{10}$ NaOH for 10 c. c. The corresponding maximum being 4·5 to 7·0 c. c. $N/_{10}$ NaOH for 10 c. c. and the acidity came down to 1·7 to 3 c. c. $N/_{10}$ NaOH.

It was also found that when the temperature at which inoculation was done was high or if the temperature rose after inoculation to 41°C. the fermentation would stop at the maximum acidity although a sample withdrawn from the vat and incubated in the Laboratory would ferment through normally.

The fermented mash was then pumped to beer tanks and distilled through Blair Campbells triple column stills. The acetone obtained was between eighty-five to ninety-five per cent. but did not pass the Indian specification. The butyl alcohol was salted out with common salt which was packed in barrels arranged one over the other. The loss on the still was brought down to 1·5 per cent. on the estimated quantity of acetone produced in vats.

The spent wash was run through underground drains to two pumps where it mixed with the household sewage and the wash water from the factory. The mixed effluent was pumped on to a sewage farm which was especially constructed for its disposal. The black soil was removed and the semi permeable murum was exposed. The excavated black soil was piled up to form bunds. Thus the whole field was divided into large shallow murum channels and wide black soil bunds. The latter were used for growing various crops irrigated by the effluent, while the channels were used for disposing of the surplus quantity by percolation.

The most important point in the process is the maintenance of pure culture of the B. Y. organism. Care was taken to keep the cultures always vigorous by examining the spore tubes before starting a culture. No trouble was experienced from this source. The pails and the seed pots also gave us very little trouble.

The infection which was found in several of the vats that failed was of two types, dipplococci and B. Voluntans mostly derived from the raw material, fine particles of which floating in the air finding access to the inside of the vats or through the insufficient cooking of the flour in the cookers. The other kind of infection which got in was from the water. These were peculiar fat rods but were found to be harmless. But when the infection from the raw material once got in it was hopeless to recover the vat. It is impossible to attain perfectly sterile conditions on a large scale, although all attempts are to be directed to achieve that ideal.

With experience it was a usual practice to keep all the lines through which sterile mash or inoculant passed always under steam and the cooking also was done for a longer time. Sterile mash was withdrawn from vats before inoculating and incubated in the Laboratory to trace the source of infection.

Slight modifications had to be introduced in cooking and grinding according to the nature of the raw material. It was found that a very coarsely ground flour could be cooked equally well with the same steam pressure as a very finely ground charge. This relieved a great deal of pressure of the mill.

It was also found that specially cooled water was not at all necessary for the acetone condensers, water of 25° to 28°C. would be quite satisfactory. Thus the power used for driving ammonia compressors was saved.

The yield of acetone on dry flour varied from 7·8 to 9 per cent. according to the quality of rice used. The yield of 90 per cent. butyl alcohol was slightly more than twice the quantity of acetone.

A great many problems have yet to be tackled in this process both from the scientific and commercial points of view. As the factory was being worked only to about one-third its full capacity it is not worth while going into the question of costs although every possible means of economy was practised.

From September 1919 to the close of the operations i. e., January 1922 a total number of 395 brews were carried out, leaving aside the 14 brews of trial period during 1919, 224 vats were fermented during 1920 of these 7 were complete and ten were partial failures. From January 1921 to January 1922, 157 vats were fermented. In the first seventy of these one was complete and five were partial failures. Then followed a unique period from May 1921 to January 1922 during which as many as eighty-seven successively successful brews were carried out without a failure of any kind. To achieve this all the probable causes of infection were eliminated as far as possible and all the operations especially of sterilisation and cooking were scrupulously supervised. At the end of January 1922 the manufacture of acetone was stopped. The Factory was sold to Bombay Government in March 1922 for being converted into a Distillery.

XVIII

THE MOHUA SPIRIT—FROM THE FLOWER TO THE MOTOR.

BY

N. N. Inuganti, G. M. V. C.

INTRODUCTION.

IT is an admitted fact that the diminishing supply of coal and oil from the fields is necessitating the attention of the economists to supplement them by other fuel. Attempts have been made all over England, France, America, Germany and other places to substitute alcohol for petrol as motor fuel with a fair amount of success either with the necessary alterations to the Engine or with the addition of other constituents to alcohol to bring it up to the level of petrol in certain respects. Alcohol is made either from vegetable sources or by synthetic process but to the economists the vegetable material is far more profitable and attractive. From time immemorial alcohol has been produced from food-stuffs such as potatoes, rice, jawar and other starch containing cereals, jaggery, molasses, etc., but under the existing circumstances these have been found in India far too expensive as raw materials for the production of alcohol to serve as motor fuel.

THE MOHUA TREE.

Apart from these raw materials there is a most useful tree that bears flowers and fruits which can be used as raw materials in the manufacture of alcohol for industrial purposes. Of late, special attention is being paid for the commercial utilisation of these flowers. This tree is known as the mohua tree (Bassia Latifolia and Bassia Longifolia) and is not unknown to the people since a very long time.

Every part of the tree is brought into use; for instance, the leaves when boiled in water are found to be a good stimulating embrocation, the bark extract a remedy for itches and rheumatic affections, the flowers mixed up with milk a cooling, nutritive, and a general tonic, the spirit distilled from the flowers a powerful diffusible stimulant and appetiser and the dried flowers are used as a fomentation in certain swellings. The seeds yield a thick oil, used externally in skin diseases, for candle and soap making and sometimes for edible purposes. The oil cake is burnt to fumigate and kill insects and other pests. The flowers are sometimes used as staple food by the poor especially during famine and more often as raw material for the preparation of country liquors by natural fermentation. This tree is found in large numbers in Bengal, Bombay Presidency, and H. E. H. the Nizam's Dominions, specially in Nizamabad and Medak districts and certain other provinces in India. As a matter of fact, tons of flowers are wasted every year and steps are now being taken to exploit them for the production of potable spirit.

Analysis of flowers:—We are concerned only with the flowers as our raw material since they contain sugar. The flowers when fresh are fleshy, juicy and cream coloured having a sweet taste and no unpleasant odour. The fresh flowers from Medchal on different days gave the following Percentages on dry matters.

Total Solids.	Glucose.	Sucrose.	Total Invert.
29	60	17·7	79
32·86	46·6	29·9	78·18
30·25	62·9	26·2	90·4

On storage they darken due to an oxidase and vary in the percentage of different sugars. The study of various sugars present in fresh flowers and the changes accompanying storage for different periods,

is in itself a problem for research. Dried flowers of Hyderabad of different samples gave the following results on analysis :

PERCENTAGES ON DRY MATTERS.

GLUCOSE	SUCROSE	TOTAL INVERT.
49·59	31·04	80·64
54·14	24·79	80·24
49·37	26·37	77·15

(*Ref. J. I. I. S. Vol. III Part VI.*)

THE MANUFACTURE OF ALCOHOL.

The process of the manufacture of alcohol from vegetable raw materials may be divided into the following five heads.

1. Preparation of the raw material.
2. Saccharification or the transformation of the starchy material into fermentable sugars by hydrolysis.
3. Fermentation or the conversion of this sugar and other sugars into alcohol with the help of certain micro-organisms.
4. Extraction or the recovery of alcohol from the fermented mass by distillation.
5. Rectification or the purification of this distillate by certain methods and the recovery of stronger alcohol.

With modern machinery, the fourth and fifth stages are combined whereby strong alcohol is obtained direct from the fermented liquors in one operation.

I. Preparation of the material :—Three methods may be employed for the preparation of these flowers.

(*a*) Flowers soaked in cold water in entire condition.

(*b*) Flowers crushed and heated in water.

(*c*) Extract taken from the flowers by boiling in water and straining.

The method of crushing the flowers and making an infusion with hot water works out the best for fermentation. The flowers should be left in the infusion during fermentation. This method also helps in sterilising the substance before fermentation. Very satisfactory results were obtained by this method and it is possible to have recourse to such a method in large factories on commercial scale.

II. Saccharification.—Mohua flowers do not require this process since the carbohydrates found in them are in a fit condition for fermentation.

III. Fermentation.—The third stage and the most important of all is the fermentation process on which lies the entire success of the manufacture.

The conversion of sugar into alcohol is done by certain micro-organisms belonging to that branch of the plant kingdom called fungi. Micro-organisms may be grouped into three classes, viz., bacteria, yeasts, and moulds. Bacteria and moulds do not produce alcohol or very little. Hence it is considered best to carry on fermentation process only with yeasts, giving no chance for either bacteria or moulds to grow on the mass.

The Mohua yeast :—Ordinary brewer's yeast or any other yeast does not satisfactorily ferment a sterile solution of mohua flowers and it is found that only the original yeast present in the flowers can ferment the mohua solution completely. In fresh fermented flowers, two principal varieties of yeast cells are found on microscopical examination one cell is found to be round in shape measuring 8 to 9 μ in diameter, the protoplasm granular, clear, and bright resembling fat globules and is surrounded by a cell wall of cellulose. Reproduction takes place by budding. The

number of spores is generally four. These belong to the *Saccharomyces cerivisea* class. The other form of cell is found to be elliptical or pear shaped, rarely elongated, usually having five or six spores. These belong to the *Saccharomyces ellipsoidus* group. A third variety is sometimes found in old fermented mashes, whose cells are more or less elongated or sausage shaped and give rise to six or eight ascospores in a cell. They belong to the *Pastorianus* group growing on the surface of the medium and giving rise to the formation of a film or scum on the surface.

Having found the two principal varieties of yeast on the mohua flowers, pure strains of them were obtained after repeated cultures and subcultures according to the Hansen's method. Experiments were next performed to see the yield and the quality of alcohol obtainable from mohua flowers; at the same time studying the conditions for a proper and satisfactory fermentation. Experiments were done under sterile conditions and with pure cultures of the two selected yeast cells. As is generally the case with organisms, these mohua yeast cells are found to grow best only on its own media, viz., mohua-peptone-agar. They can be trained to grow on other media but when habituated to any other media they lose the virulence of fermenting mohua solution satisfactorily. Two experiments under similar conditions were conducted, viz., (1) a known quantity of mohua was fermented with a pure culture of yeast cell and kept sterile throughout and (2) the same quantity left for natural fermentation (open), other conditions being similar in both cases. The pure culture fermentation gives a higher yield than the other and by the addition of certain substances such as ammonium phosphate, ammonium sulphate, sulphuric acid, etc., a still higher yield is possible to the extent of even 91 per cent. of the theoretical yield. (*Vide Table p. 118.*)

Quantity	Other constituents	Fermentation started in	Fermentation completed in	Distillate obtained	As absolute alcohol	Calculated on theoretical yield
100 gms. Mohua taken	Spent wash 200 c.c. Dilute H_2So_4 1 drop	10 hours	3 days	900 c.c. of ·9640 i.e., 31 per cent.	27·9 c.c.	72·5 per cent.
	Spent wash 200 c.c. H_2So_4 2 drops	6 hours	30 hours	51 .cc. of ·9217 i.e., 56 per cent.	28·5 c.c.	74 per cent.
	Spent wash 200 c.c. ammonia phosphate. 1 gm.	5 hours	36 hours	100 c.c. of ·9630 i.e., 33 per cent.	32 c. c.	83·1 per cent.
	Spent wash 200 c.c. H_2So_4 2 drops ammonia phosphate. 1 gm.	4½ hours	28 hours	100 c.c. of ·9587 i.e., 35 per cent.	35 c. c.	91·5 per cent.

Factors for good fermentation:—High temperatures are dangerous, while low ones, though not very harmful, slow down the process. A temperature of 32°C. to 37°C. is found quite good for a regular and quick fermentation. Darkness or diffused light is better than strong intense light. The percentage of sugar in the solution to be fermented should be between seven and twelve per cent. Yeast food like the sulphate and phosphate of ammonium are useful. The addition of spent-wash plays a good part. The medium should be mildly acidified with organic acid. Alkalimety is harmful for the yeast.

IV & V Distillation and Rectification—This process is merely mechanical and with the latest type of triple effect distilling and fractionating column 95 per cent. strong alcohol can be obtained in one operation.

The Crude method of manufacture:—It may be interesting to note that crude methods of fermentation and distillation exist in certain places even to this day. The plant of an ordinary distillary is simple. From the store room mohua flowers (stocked) are brought and filled in wooden fermenting vats to a certain specified level, which means a certain weighed quantity, and water is let in to the whole mass up to a mark, (about 45 gllons to a maund), and 15 gallons of spent-wash to a maund also added, stirred, and left for natural fermentation. In 24 hours fermentation sets in if the day is not chill and continues for a few days. The distiller takes the saccharometer readings every day until it goes down to say about 4° to 3°. Then the fermented mass is pumped up into the still and steamed when dilute alcohol distills over varying in strength. This sprit is fit for drinking purposes.

If stronger spirit is needed, this has to be fractionated in a rectifying column.

Still more crude methods are adopted by individuals known as kalals as cottage industry. Mohua

flowers are fermented in wooden tubs under natural conditions and when finished to a certain stage (eye-test) they are put in copper vessels (Dekshis) with a goose neck fitted up, around which there is a large cup shaped receptacle into which water flows serving as a condenser. When the vessel with the flowers and water is heated on naked fire hot distillate comes over which is collected, cooled, and stored up for issue. This is naturally a highly diluted alcohol but is in enough proportion for drinking purposes. By such crude methods less quantity of alcohol is invariably obtained due to evaporation for lack of proper condensation and more so to the presence in the fermenting substance of other organisms and moulds which retard the process of regular and satisfactory fermentation.

Possibilities of alcohol as motor fuel :—It is to be considered if alcohol can be used as a satisfactory fuel in motors in place of petrol, and if so whether by itself or by the addition of any other subtance. Since, of late, alcohol has been used for driving motors and engines in Norway, Germany, and other places, the first attempts to substitute alcohol for petrol was made in a petrol engine by Hartmann who reported that the characteristics of combustion were better and inodorous and that it consumed 839 grams of alcohol against 426 grams of petrol per H. P. Later on, Berlin Fermentation Institute made a few trials. At the same time Slaby of Charlottenberg Polytechnic tested in 5 H. P. motor and obtained per H. P. a consumption of 550 grams of alcohol of 86·2 per cent. Hank tested a 6 H. P, motor internally fitted up for the use of alcohol and obtained a force of 9·93 H. P. with a consumption of 390 grams of 93 per cent alcohol per H. P. The first locomotive of 15 H. P. was tested and 21·8 H. P. obtained with a consumption of 410 grams of 88 per cent. alcohol mixed with 20

per cent. benzol. These foregoing tests by various workers prove that alcohol, whether by itself or mixed with other substances, can advantageously be employed for motive power and can replace petrol.

Advantages of alcohol as fuel :—The use of alcohol has got advantages peculiar to its own.

1. The safety of alcohol as compared with petrol in not forming an explosive mixture at ordinary temperatures and the ease with which its fire can be extinguished, its missibility with water, makes it a less dangerous fuel than petrol.

2. Alcohol contains in its molecule a large proportion of oxygen necessary for its complete combustion. Hence alcohol requires only about one third the amount of air required by petrol. This fact accounts for the high thermal efficiency obtainable with alcohol. The products of combustion are carbon dioxide and water, and do not produce any smoke or foul odour as in the case of petrol, or deposit of soot carbon which acts as an impediment to the smooth running of the engine.

3. Alcohol has a greater range of explosive mixture with air than petrol, hence it causes less knocks to the engine and ensures smooth running.

Disadvantages of alcohol compared with petrol :—The low calorific value and higher flash-point, and density militate against the use of alcohol in the place of petrol. The following will show the figures.—

	Flash Point.	Density.	Calorific value for one Gram.
Petrol	35° C.	·7745	11,209
Alcohol 94%	37° C.	·8027	7245
Ether	27° C.	·7115	9807
Butyl alcohol	52° C.	·8163	9007

Hence it is sometimes difficult to start the engine when cold, and weight for weight lesser energy in

the form of heat is obtained. Again, should the combustion be imperfect acetic and other corrosive products are formed which are prone to attack the metal surfaces, causing deterioration of the engine.

Nevertheless, it is possible to remove the disadvantages. One method is to blend alcohol with other fuels to bring it up to the level of petrol or to make certain alterations in the carburetter or compression ratio of the engine or both. It is suggested to use a more volatile fuel as a starter or to mix a proportion of more volatile liquid with the alcohol itself. To make up for the lower calorific value of alcohol it is desirable to increase the fuel supply by increasing the area of the fuel supply jets and pipes. The thermal efficiency of alcohol can be made greater than petrol to counterbalance its lower density and calorific value. Thus with alcohol there is not the same difficulty in getting complete combustion, since one volume of alcohol vapour requires one third the quantity needed by petrol. This smaller dilution ensures more perfect mixture before the explosion and reduces the waste of heat in the exhaust. The mixture can be subjected to a pressure of 200 pounds per square inch in the cylinder without spontaneous ignition, whereas the safety limit with petrol is only 80 pounds. The efficiency per brake horse power with alcohol is 28 to 31 per cent. compared with 16 to 22 per cent. with petrol. The higher efficiency compensates for the lower calorific value and density of alcohol. To prevent acidity during combustion, the mixture can be made alkaline with ammonia to neutralise any acids formed in the exhaust gases. By a series of experiments it was found that about 20 per cent. of ether may be retained in alcohol below 45° C. and 10 per cent. butyl alcohol can come over at 80° C. Such a mixture remains homogeneous throughout combustion.

Denaturation :—Alcohol being used as a main constituent in motor spirit it is but compulsory to denature the spirit in order to make it unfit for use as beverage without in any way affecting the working of the engine. Ether itself forms one but since it could be easily fractionated substances such as bone oil which contains a large amount of pyridine, benzene, benzol, wood-naptha, spirits of turpentine etc. are added which make the spirit repugnant to smell and taste. In fact petrol itself can act as a denaturing agent as it is easily miscible with the spirit to the extent of 15 to 20 per cent. and its smell is never got rid of even by distillation.

Various mixtures :—Various patents have been taken for the motor spirit and several brands are on the market. Where there is a large production of tar, coal tar, benzene is produced which is admixed with alcohol and used as motor fuel. Acetylene gas and hydrogen have also been tried but to no success. The addition of sulphuric ether is quite favourable and the fuel '*Natalite*' of South Africa consists of a high percentage of ether (about 45 per cent.) mixed with alcohol together with a small quantity of ammonia and arsenious acid. As ether could be easily manufactured from the produced alcohol, these two, in a good proportion together with some denaturant will form a suitable and cheap substitute for petrol as motor fuel as the question of cost plays an important part in the concern.

By encouraging agriculturists to pay more attention to the mohua crops, by erecting plants in centres where mohua crops are in abundance to reduce the cost of conveyance, and by increasing the out-put of alcohol with improved and properly controlled fermentation, the cost of alcohol can be brought down to a low figure. This would ensure a regular demand of this motor spirit when the price and general efficiency compared to petrol is taken into consideration.

The following table may be interesting to note since these trials were made with the various mixture in a stationery motor.

No,	Constituents	Specific Gravity	Calorific value for 1 c. c. tried in tin Calorimeter	Calorific value for 1 Gm. tried in tin Calorimeter	Flash point Open test (Centigrade)	Flash point Close test (Centigrade)	Number of revolutions per minute	Quantity consumed per minute	Number of revolutions for 1 litre spirit	Observations.
A	Petrol	.7542	7120	9440	33°	26-27	700	20 cc.	35,000	Flame smoky engine steady running and uniform working.
B	Alcohol 90°/₀ 78 Butyl Alcohol 9 Ether 13	.8025	5665	7059	30°	26°-27°	700	25	28,000	Flame not smoky-steady running when there is constant supply
C	78 9 13 Saturated with acetylene gas	.8102	5665	7016	28°	25°	755	30	25,166	Flame not smoky-uniform running when there is constant supply.
D	80 : 20	.7805	5670	7223	28°	25°	717	24	20,875	Smokeless flame. Good start & easy running requires less air.
E	Alcohol 90°/₀ Ether. 80 : 20 Saturated with acetylene gas	.7179	5692	7224	27°	25°	800	25	32,900	Smokeless flame. Steady run as long as there is contant supply

XIX

THF FLAVOURING OF EDIBLE OILS.

BY

Arthur J. Solanke, B. Sc.

WITH the disappearance of hand looms and cottage-industries, and with the advent of machinery and steam engines, the question of food supply in big centres of industry in Europe, became very acute. During peace time, however, the question of food supply is not so pressing as during war and it was the "War Lords of Yore" who directly stimulated the manufacture of artificial foodstuffs or more properly food substitutes, chief among which was margarine, the substitute for butter.

Margarine was originally a war-product created by the Franco-Prussian War and it may be said to have now established itself as a common article of diet as a consequence of the recent World War. Long before the Franco-Prussian War, Napoleon III had offered a prize for the successful production of a fat which should be as appetising, nutritious and stable as butter, and in 1870, Mege-Mouries secured the prize for his invention of oleo-margarine. He prepared this margarine from beef-fat, but as time went on various fats were used by different manufacturers and during recent years hydrogenated vegetable and animal oils have been extensively used in the margarine industry.

The margarine prepared by Mege lacked taste and he therefore sought to improve its flavour by churning the oleo-margarine with 10 per cent. of cow's milk, and water containing macerated cows' udder (0·4 per cent.) The resulting emulsion was solidified, washed,

salted and coloured and was sold as a butter substitute. In principle the method of Mege is followed even now, one very important improvement introduced in the method being the use of pure lactic acid organisms flavouring the milk used in the emulsification of the fat. During recent years artificial milk prepared from seeds of edible oils, especially soya is used.

The principles underlying the flavouring of oils and fats will be well understood if we examine two of their chief properties. The first property is that a fat or an oil easily absorbs smell, either good or bad, from a substance with which it comes in close contact. This is well illustrated from an instance of cream which is prepared from foul smelling milk; not only cream, but the butter prepared from it gives the foul smell.

The second property is that fats and oils become hydrolysed when exposed to light, air or moisture and gives the well-known rancid smell of butter or cheese; there are certain micro-organisms also which hydrolyse the fats and oils.

The first property of the fats and oils mentioned above is very important because it enables one to impart the desired flavour to them. It is essential that the materials used in flavouring oils and fats are of the very best quality devoid of any smell excepting the desired one. The oils used must also be odourless and flavourless; only then can complete success be attained. The problem is essentially that of the perfumer. He extracts the perfumes from flowers by means of pure odourless fats; we can do the same with regard to edible fats and oils by bringing them in contact with well flavoured milk or other suitable media.

The second property mentioned above is also an important one. Once good margarine is made it is not an easy matter to keep it from going bad. The flavoured fat should not be exposed to sunlight,

moisture or air; consequently it should be stored in closed metal vessels. It has been the custom in India from times immemorial to store ghee in earthern vessels. To prevent the activity of micro-organisms a suitable preservative should be used, 2 per cent. salt being the best preservative from all points of view.

In India, the question of flavouring oils and fats is a slightly different one. Indians do not regularly eat butter as they do ghee which is prepared from butter by heating it till all water has evaporated from it; so ghee is nothing but pure animal fat obtained from the milk of a cow or buffalo, Fresh, genuine butter has a mild pleasant odour but when it is heated to produce ghee, quite a different odour is developed, probably depending on purely chemical action. It develops when the butter is being heated and it may not be impossible to reproduce the ghee smell from the constituents of milk by purely chemical reaction.

XX

UTILISATION OF INDIGENOUS COLOURING MATERIALS.

Rausaunt Wood (Indian Barbery, Barberislycium.)

BY

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INTRODUCTORY.

INDIA, with its numerous mountains and rivers, is naturally suited for luxuriant vegetation, the growth of which is accelerated a hundredfold by the tropical sun. It abounds in large quantities of vegetable raw materials which find various medicinal and other economic uses. With the advent of synthetic drugs and dyes, the employment of the natural ones is becoming more and more restricted.

The great war demonstrated the economic dependence of India on other countries, when the supply of the synthetic dyes and medicines being cut off, enquiry into the utilisation of the various Indian colouring matters was stimulated. The first to suggest itsself, on account of its abundant occurrence and extensive usefulness in arts and medicine, was the ***Rausaunt wood or the Indian Barbery.***

DESCRIPTIVE.

The plant belonging to the natural order ***Barberridace*** is a perennial thorny shrub found in the Himalayas and Nilgiris. Its branches are arched, hanging at the ends, and bears elegant dropping leaves and yellow flowers. The berries are small, red or violet oval or oblong according to the variety, and contains two or three seeds.

HISTORICAL.

The drug according to the Indian pharmacopeae finds employment in ophthalmia, ulceration, malaria dyspepsia, rheumatism and sun-blindness. It is also prized as a dyestuff on account of its yellow colouring principle.

CHEMICAL COMPOSITION.

The active principle of some species of the Barberridacae seem to have been investigated by many and found to be an yellow coloured alkaloid, barberine. Other plants of the order *Ranunculaceae*, *Manispermaceae*, *Rutaceae* &c., also have the same principal alkaloid. Chavallier and Pellitan detected this alkaloid in prickly ash in 1826 and Buchner noted in common Barbery in 1835. It has also been detected in *Barberis*, *Aristata*, *Hydratis anadensis*, *Coptis tota*, *Barberis*, *Aquyfelium* and *Iodolia Aquyleta* and a host of others by many workers. The alkaloidal nature of barberine was first determined by Fleetman and Perrin and its constitution mainly elucidated by W. H. Perkin and Gadamer. An examination of the plants of the species given above has been so far made whilst other species of Barberis of Indian Flora do not appear to have been investigated. In the present work Barberis Lyciam (supplied through the courtesy of the Forest Officer, Kangra) has been examined for the chemical nature of the active principle contained in it and the study of the dyeing properties.

The investigation was divided into two parts:—

PART I.

(A) Preliminary observation for various organic substances present in the wood. (B) Extraction of alkaloids by different solvents. (C) Detection of the alkaloids by different colour tests following "Fuller and Dragendorffs' scheme" (for the detection of the alkaloids). (D) Isolation and determination of the chemical nature of the principal alkaloid and preparation of its salts.

PART II

(*a*) Dye trials with the colour-base extracted from the wood.

(*b*) Determination of fastness of the colour.

(*c*) Production of different colours with other indigenous meterials and attempts to substitute it for materials not procurable in India.

(A) Preliminary observations were carried on with a view to determining the nature of some of the organic constituents present and it was found that the aqueous extract contained volatile substances, fixed oils, alkaloids, sugars, glucosides, organic acids, colouring-matters etc., in the following proportions:—

	PER CENT
Volatile substances	1 to 2 „
Fixed oils	0·25 „
Resins	1·7 „
Organic Acids etc., precipitated with Lead Acetate	15 to 18 „
Sugars	2·02 „
Tannins	?
Brown Colouring matter ...	10·8 „
Total alkaloids	? „

The Analysis is not complete as only a few constituents were examined. Its ash content is 3·79 per cent, and moisture content 10·21 per cent.

(B) Extraction with water was carried on at different temperatures with a view to determining the Optimum temperature.

TEMPERATURE.	PERCENT EXTRACTED	TEMPERATURE.	PERCENT EXTRACTED.
23° — 25°	7·73	70° — 80°	17·21
50° — 60°	19·42	80° — 90°	20·21
60° — 70°	14·24	90° — 100°	21·51
Acidified water 23° — 25°C	16·1	Alcohol	15·33

Extraction with the following volatile solvents was carried on in the order given in a Soxhlet :—

Name of Solvent.	Percent extracted.	Remarks.
Petroleum Ether	0·25	Very slight yellow colour extracted.
Ether	0·63	Yellow colour extracted.
Chloroform	11·33	Large quantities of yellow colour removed.
Ethyl Acetate	0·19	Slightly yellow coloured extracted.
Alcohol	0·17	Slightly yellow coloured extract.

The residue was still slightly coloured and could be extracted with water. Coarse material gave better results than fine.

(C) Detection of the Alkaloids was carried on by extracting the material with alcohol and acidified water and by following Fuller and Dragendorff's schemes, the results showed the presence of barberine and its associated alkaloids Oxyacanthine, Colchineine, Barbamine, Canadine, Columbin and probably Narcine. The probable glucosides were Populin, Senegin, Smilacin and Saponin. The reactions of these alkaloids as found are given in the table attached. (*Vide table No. III*).

(D) Isolation and indentification of the principal alkaloids were carried on by purifying the acidified aqueous extract with basic lead acetate and then precipitating it with potassium iodide, dissolving the precipitate in amyl alcohol, breaking it with thiosulphate and extracting with alcohol. The other method for purifying them was by smilarly extracting it with acidified water and proceeding as before making it alkaline with ammonia and evaporating until all the alkaloids crystallised out from the concentrated solution. The molecular weight of the alkaloids was determined by analysing the gold salt of the base.

0·2452 grammes of the Auric salt on ignition gave 0·0716 grammes of gold corresponding to 29·22 per cent.

$(C_{20}H_{17}NO_4)$ $HAuCl_4$ requires 29·16 per cent. Nitrogen by Kjeldhal's process amounts to 3·79 per cent. Barberine, $C_{20}H_{17}NO_4$ requires 4·17 per cent.

The melting point was found to be indefinite. The alkaloid changed from yellow to brown at 100° C. sulphates, nitrates and chlorides of the salts were prepared by crystallisation; aurichloride, iodide, and chromates of the base were prepared by precipitation. The percentage of the base was determined with potassium iodide volumetrically, which amounted to 6.39.

PART II.

(*a*) Dyeing properties of the colour obtained from wood were determined by making several dye trials on cotton, wool and silk. It was found that it is an adjective dye as it did not colour cotton without the help of tannic acid. The aqueous acidified extract was used for these trials. The effect of different mordants was tried on the aqueous solution in order to ascertain which mordant will be suitable for its application on the fibres. Stannous chloride, potassium dichromate, tannic acid, zinc sulphate calcium acetate, barium chloride, etc., gave a precipitate. After these preliminary observations dye trials were carried on with a view to determining the effect of concentration, temperature and addition to the dye-bath. It was found that 15-20 times boiling water for 45 minutes to one hour gives good results and that one per cent. of sodium carbonate added to the dye-bath is beneficial, increasing the intensity of the colour towards brown without impairing its brilliancy. Full shade is obtained with 20 per cent. of the dye stuffs. Neutral bath with the addition of glauber's salt in the case of silk works better than one without it. Various shades of the colour on different fibres with different mordants were as follows:—

On Cotton:—i. Simple dyeing in an infussion of water with 20 per cent. dyestuff.

ii. First mordanting with alum, chrome alum, iron and tin salts, 2 per cent. each and tannic acid 10 per cent. on the weight of the goods and then dyeing as above.

iii. Dyeing as in (i) and then treating with 1 per cent. of chrome alum, ferrous sulphate, copper sulphate and boil for half an hour.

On Silk:—The same as on cotton.

On Wool:—The same as on cotton.

The shades obtained by these dye trials are given in the table attached. (*Vide Table No. I*).

(*b*) The fastness of the colour to light, washing, alkalis, acids, and reducing and oxidising agents are given in the table (*Vide Table No. II.*) The dyeing properties of the colour show that it is fast to washing and acid to a slight extent and is not fast to reducing and oxidising agents.

(*c*) Different greens were prepared by dyeing fibers with the colour and topping with natural indigo. The green colour thus produced was tested for its fastness and was found to be fast to washing, light, and acids and fairly so towards alkalis and bleaching agents, equalling the direct greens in shade and fastness. It was tried as a substitute for Fustic, a yellow vegetable colouring-matter imported into India and largely employed for the fast vegetable blacks and navy blues with logwood on the fibre, espicially wools. It was found to serve the purpose giving a deeper shade and equalling it in fastness and at the same time being cheaper. Alcoholic extracts containig the resinous matter were found to give a very good brown of great fastness.

SUMMARY.

I. These experiments show that—The wood contained volatile substances, fixed oils, sugars, glucosides, resins alkaloids, etc.

II. Barberine is the principal alkaloid amounting to 6·39 per cent. Other associates detected are Oxyacanthine, Oolchicine, Barbamine, Canadine Columbin and probably Narcine which affect the medicinal properties of the extract. The amount is 4·41 per cent.

III. A large proportion of the material is extracted with at 50—60°C. Amongst the volatile solvents, alcohol and chloroform give good results.

IV. The dyeing properties of the colour show that it can mostly be used for silk and to a limited extent for cotton mordanted with tannic acid. On silk pure and bright yellow colours are obtained.

V. The colour can be used for preparing green shades of good fastness by combination with indigo. It can also be used as a substitute for fustic and log-wood dyeing. Brown shades of good fastness can also be obtained from it.

TABLE No. I.

Table showing the shades obtained with the colour on differently treated fibres.

Numbers	1	2	3	4	5	6	7	8	9	10	11
Reagents for treatment of Fibres	Simple dyeing	First mordanting and dyeing					Dyeing and after treating with			Iron Sulptate	Turkey red mordant
		Aluminium Salts	Chromium Salts	Copper Salts	Iron salts	Tannic acid	Alum	Potassium Dichromate	Copper sulphate		
Cotton	Yellow	Yellow	Greenish yellow	Greenish yellow	Reddish yellow	Deep brownish yellow	Faint yellow	Yellow	Greenish yellow	Reddish yellow	Pure yellow
Wool	Dull yellow	Dull yellow	Reddish brown	Brown	Reddish brown	Dark brown	Brownish yellow	Brown	Brownish yellow	Reddish brown	
Silk	Pure yellow	Dull yellow	Brownish yellow	Greenish yellow	Deep yellow	Deep yellow	Greenish yellow	Brown	Brownish yellow	Brown	Pure yellow

TABLE NO. II.

Table showing the fastness of the shades obtained with the colour on fibres.

Fibres	The No. of shades as given in the table of shades	Light	Boiling water	1 % Soap solution at boil	Acids 10 per-cent acetic-acid	Alkalies 5 per cent sodium carbonate	BLEACHING AGENTS 1 percent Hydro sulphite solution	1 Per cent bleaching powder in the case of cotton and Potassium permanganate and sodium peroxide in the case of wool and silk
Cotton	1	Not fast	Not fast	Not fast	No special effect. In some cases the yellow colour gets dissolved and thus shade becomes decreased in intensity.	The colours change to brown. The shade can be reclaimed by treating with acids.	The reducing agent in this mild form in the cold has no action.	Not fast
	2	"	"	"				"
	3	"	"	"				"
	4	"	"	"				"
	5	"	Fairly fast	Fast				"
	6	Fast	Fast	Fast				Fast
	7	Not fast	Not fast	not fast				Not fast
	8	"	"	"				"
	9	"	"	"				"
	10	"	"	"				"
	11	"	Fast	Fast				"

Wool	1	Not fast	Not fast	Not fast	The Shade becomes Yellowish in tone in all cases as well as decreased in intensity ; it kept for a long time.	The shade becomes brownish.	The colour is not effected in neutral solutions	The colour is not effected tn neutral solutions.
	2	„	„	„				
	3	fast	„	„				
	4	Not fast	„	„				
	5	„	„	„				
	6	Fast	„	„				
	7	Not fast	„	„				
	8	„	„	„				
	9	„	„	„				
	10	„	„	„				
Silk	1	Not fast	Not fast	Not fast	Ditto as in the Case of wool	Ditto as in the Case of wool	Ditto as in the Case of wool	Ditto as in the Case of wool
	2	„	„	„				
	3	„	Fast	„				
	4	„	Fairly fast	„				
	5	„	Fast	Fast				
	6	Fast	Fast	Fast				
	7	Not fast	not fast	Not fast				
	8	„	„	„				
	9	„	„	„				
	10	„	„	„				

N. B.—The numbers denote the shades as given in the dye-trial table.

TABLE

Table Showing the Reactions of the alkaloids in Dragendorffs' scheme.

Name of the extract	Concentrated sulphuric acid	Concentrated Nitric acid	Myers reagent	Picric acid	Frohde's reagent
1 Precipitate with potassium iodide dissolved in sodium thio sulplate	Reddish brown colour	Orange red changing to reddish violet colour	Yellowish white precipitate	Yellow crystalline precipitate	Violet changing to blue
2 Acidified petroleum	Reddish brown colour; changing to violet green	Ash colour	do	Crystalline whitish yellow precipitate	Yellowish brown colour changing to green
3 Acidified benzene	Reddish violet colour deepening with nitric acid	Dark red colouration	Whitish flakes	Yellowish white precipitate	Violet colouration
4 Acidified chloroform	Deep reddish brown colour	Brown colouration	Yellow flocculent ppt.	Deep yellow granular ppt.	Do
5 Ammoniacal petrolium ether	...	...	...	...	...
6 Ammoniabenzene	Brown to orange brown colour	Yellow colouration	Yellow ppt.	Yellow granular ppt.	Violet coloration channging to blue black
7 Ammoniacal chloro form	Yellow portion brown white portion no coluor	Yellow portion brownish	Yellowish white ppt.	Yellow crystalline precipitate	Deep violet colour changing to green
8 Ammoniacal Amyl alcohol	Yellow colour; adding nitric acid reddish brown	Reddish brown	Yellow ppt. on standing	yellow ppt.	Violet Colour Changing to green

No. III.

different solvents of the Ransant wood according to

Chlorine water	Potassium iodide, and Sulphuric acid or tannic acid	Ferric choride and Sulphuric acid	Potassium dichromate and sulphuric acid
Red colouration at the contact	With tannic acid brown ppt.	...	...
...	...	...	...
...	With tannic acid Yellowish white ppt.	Reduced to dirty green colour	Reduced to green colour
Red fluores-cence	...	Dirty green changing to brownish red colour	...
...	...	...	...
Yellowish colour	Brownish yellow ppt. with both	...	...
No special colour Orange on adding sulpuric acid	Iodine evolved	Green colour	Red colour
Orange colour	Iodine evolved	Greenish red colour	...

XXI

AN INTERESTING APPLICATION OF MODERN METHODS TO A PRIMITIVE INDUSTRY

BY

Yeswant Dattatreya Wad, M.A., M.Sc., A.I.I.Sc.

THE use of Katha in chewing with "pan"-betel-leaves is an ancient practice in this country. Katha is normally a pinkish white crystalline substance with an earthy fracture and composed chiefly of Catechin. It is extracted from the heart-wood of the Khair tree–*Acacia Catechu* and appears on the market, in various forms, the purest being generally two inch cubes or round lumps of corresponding size. The many grades that are found on the market are due to local differenences in the methods of manufacture and frequently to adulterations practised by the dealers, thus presenting the basic substance catechin—in admixture with natural and artificial impurities. Mr. Percy Brown in his monogragh "Notes on Cutch", gives all details about the commercial varieties and the methods of their manufacture. The principal natural impurity is Cutch, the dyeing principle of the Khair, the heart wood of which always contains katha, and so derives its characteristic colour, The dyeing properties of this second substance are also known from a very old date, and Cutch is still used as a dye in India. At present, owing to artificial dye-stuffs coming into common use it is naturally not used on a large scale except perhaps for colouring fishing-nets, which it makes more resistant to sea-water. It is also used to a small extent as tannin. In spite of the fact that both these substances were

in use since a very ancient date in this country it appears that they were always considered to be one and the same substance, the two different names being due to provincial causes and do not denote any attempt to differentiate the separate constituents of the tree. They have now acquired a definite meaning—katha being that part which is very rich in Catechin and Cutch that which is rich in the dye. This allocation of names is not empirical but has a natural basis. The product of most Indian Khairs is richer in Catechin and the Indian name for that is Katha hence it is convenient to restrict the term for the substance rich in catechin. The Burmese product—the Cutch of commerce—is richer in the dye and hence that term should properly be reserved for the dye. This difference in composition in the products, although to some extent due to differences in the manufacturing methods, appears also to depend on the initial proportion of Catechin to the dye in the tree itself, as well as the amount of a third substance the Khair gum. This alone is sufficient to yield products of widely varying composition even when the same process is employed for the extraction of wood of a different origin. The inadequate realisation of this fact has often led to great confusion while applying modern methods to the manufacture.

At present the consumption of Katha in India is solely for chewing purposes and to a small extent for medicine, while Cutch the dye is exported to Great Britain for the use of Scottish fishermen.

Old Method of Manufacture:—Like every other thing in India, this Industry is still carried on in the same ancient manner by the same ancient people. The middlemen may have changed but the manufacturers remain the same hill tribes in whose home jungles the Kahir abounds. They are named differently according to locality for instance

Kanthakar-makers of Kantha-in the Bombay Presidency and Khairis on the Himalayan slopes. The manufacture is undertaken as piece work by the families of hill-men from merchants who possess monopolies over the forest. The details of the manufacture are arranged by the families themselves who divide the process into two parts; the first stage requiring hard work, is done by the men and the subsequent processes of extraction etc., are in the hands of women. Thus it is the women of the tribes that really know how to make Katha or Cutch. Such a division of labour is found in many other Indian trades.

Although a detailed account of the process is beyond the scope of this review, it may be of some interest, at this stage to give a brief outline in a general way omitting local differences in detail. As is usual with everything in a forest, the making of Katha is done after the rains are over. The dry winter is specially preferred as the properties of the product make the manufacture difficult and uncertain except in the cold weather. The tribesmen are totally ignorant of this, but they follow established usages accompanied by much superstition. Most of the differences in detail in different localities when closely examined are found to be the natural result of the combined effect of climate, the nature of raw material and the means available. The heartwood is only taken and from that too only those pieces which their expert eye selects as suitable are kept, the rest being used as fuel. The men go to the forest, fell trees and chip the heartwood to small pieces about an inch in length. The chips are then extracted by women in a battery of earthenware pots kept over a mud furnace. The liquid in the pots is transferred from one to another in such a way that every lot of fresh chips is extracted five or six times. The extracted chips are then used as fuel. The dark red extract is concen-

trated to the required thickness in a special pot reserved for that purpose and then poured into moulds or pits whose bottom is generally covered with some porous material as sand, straw, rice-husk ete., according to locality and also covered with straw, bamboo mats etc. After a few days it is expected to set when it is taken out and given the requisite shape. In Burma they use iron kettles for evaporation and the concentrated extract is poured directly into moulds. Sometimes a whole lot goes wrong and does not set properly when it is generally attributed to supernatural causes. The merchant can only interfere at the last stage and if so inclined introduces adulterants like flour, sand according to his needs. The makers themselves do not adulterate and the impurities in their products are inherent to their process.

It is quite obvious that this procedure leads to a tremendous waste as all the wood containing Katha and Cutch is not extracted some part being rejected. No attempt is ever made to prevent loss of the Cutch as the main idea is to get a solid Katha. Only in the Burmese product a large amount of the dye happens to be retained. Nothing is done to restore the destroyed Khair trees. The out-put at any one place must necessarily be small and the manufacture has to be carried on in the heart of jungles and only at certain seasons when there is ample water available and the requisite weather conditions exist; failing these, the product can never be of any standard quality.

The Chemistry of the Khair tree and its products has been studied to some extent by Dr. Warth and others, but nothing was done to study the subject with a view to placing the industry on a sounder basis until very recently when the United Provinces Forest Department began attempts to develop forest industries such as turpentine manufacture. They wanted to

utilise the the vast Khair forests in the Kumaon and gave facilities to Mr. Mirza, Manager of a tea-garden near Dhera-Dun for working at the problem in their laboratories. These experiments led him to open a small factory at Ramnagar near Naini Tal. Though it subsequently proved a financial failure a great deal of valuable data for large scale work was obtained. Thereafter the Indian Wood products Co., Ltd., Barielly, Izatnagar, United Provinces manged by Messrs. Gillanders, Arbuthnot & Co., Calcutta, was started to take up and perfect the work at Ramnager on a sufficiently large scale. Preliminary experimental work was done by Mr. Mirza, the Ramnagar Expert, Dr. Perkin and also at the laboratories of Messrs. Kestner and Co., in England and a modified process was adopted for the Izatnagar factory based on the data thus collected. The writer of this paper joined the concern just when the process was being given the first trials. Subsequent experience led to the discovery of unforeseen difficulties which had to be overcome by introducing suitable modifications.

The Khair Trees:—Before following the development of the present process from the Ramnagar Factory, let us know more about the Khair tree. It is found in large quantities in the forests of Burma, Himalayan Slopes and the Western Ghats. Normally it exists scattered except in a few places such as the Terai and the Burma Jungles where it is found gregarious and possessing gigantic growths. It is of no particular value as timber and hence is not given any special attention by the the forest authorities. It varies much in size according to environment from a small pigmy to gigiantic growths, the trunks, sometimes measuring more than 4 ft. in diameter. The bark contains only a gummy substance, the percentage being the greatest in younger parts than in the older ones. The gum content also decreases with the time elapsed after a log is cut and left in the yard under the

ordindary atmospheric influences probably due partly to fermentation in the green log which sometimes contains 40 per cent. of moisture, and partly to oxidation. The amount of this gum has a very great influence on the behaviour of the extract in a Cutch factory as under factory conditions it is necessary to use whole logs and not simply heartwood. The amount of each component and their relative proportion varies much. The highest total solids found being 22 per cent. and the low figure of 10 per cent. is not infrequent. The Katha-catechin—also varies between 2 and 7 per cent. An average of 15 per cent. however is taken as normal. It appears that a tree growing in rich soil in the plains and in moist atmosphere contains more cutch and probably gum also, while when growing on hill-slopes enjoying—as in the case of the Western Ghats—comparatively dry weather conditions during the greater part of the year, the Katha or Catechin is found in greater proportion. The Katha is sometimes found in nature deposited in cracks and between fissures of the stem in the form of a pink crystalline powder which is called Khersal ; it is supposed to possess special medical properties and fetches very high value.

Properties of Cutch:—The Cutch or dye of the Khair wood is very soluble in water and is not a crystalline body. In solution it has a dark red colour and gives a fast dye when oxidised and mordanted. The solid has the same colour except on contamination with iron, when it is dull black. The solid has conchoidal fracture. When once properly dry and free from gum, it is not affected by heat and resists the action of moisture to some extent as the water does not easily penetrate the mass. This latter property is due to the fact that solutions of Cutch of different strengths do not mix easily. When, however, the gum content exceeds certain limits the solid mass behaves like pitch on exposure to heat or sun, becoming plastic and melting.

In this state it is highly inconvenient to handle and has given much trouble everywhere, leaking through packing cases, attaching them to each other or to the floors of the store, railway waggons and dock yards, making such a mess that for sometime it became the business of every body concerned to find a way out of the difficulty. The dyeing power of this stuff varies according to the process used for the preparation and seems to depend on the percentage of actual colouring principle which is perhaps an oxidised form of the basic substance in Cutch. The production of this active form is enhanced by heat alone or in conjunction with air, the latter conditions being more favourable. This treatment gives a darker colour, a pumice-like vesicular structure, it becomes very friable, more resistant to moisture and is unaffected by exposure to heat or sun. It can then be readily packed in ordinary gunny bags and gives no trouble in transport. As a dye it is more effective and is less soluble in water. Due to the peculiarities of the process, Burma Cutch contains this form in larger quantity and hence is always bone-dry and possesses immense dyeing power, while machine-made Cutch does not always possess these properties. The cause was unknown until very recently when the author succeeded in making Cutch of a higher dyeing power than that of the Burma product. It is now certain that even machine-made Cutch, if properly made, should be capable of being packed in bags and giving no trouble in transit; it can be so made as to possess tinctorial value higher than that of the crude Burma material.

The properties of Katha.—The other component Katha or Catechin is a pinkish white, crystalline body, difficultly soluble in cold water. This makes it possible to separate it on a large scale from the accompanying Cutch by cooling a supersaturated solution to a moderate temperature in practice between 60° and

70° F. The crystallisation is retarded by the presence of undue quantities of the Khair gum which is present when whole logs are used in green condition. The green ferments in the crystallising tanks and the frothy liquor cannot be properly filtered. The separation of Katha and Cutch is incomplete and this leads to great trouble both in the absorption and drying chambers the Katha produced is mouldy and unsaleable. The crystallisation is also retarded if the proportion of Katha to Cutch is small probably because the solution has to be made very thick, before the saturation point for Catechin is reached. In many cases, seeding with Catechin has started the action. The crystal mud, after separation and washing, requires rapid drying or it may become mouldy and unfit for use. The final appearance of the article depends on its purity and also on the speed of the period of drying. The purer the material the easier it is to dry. The lower the drying temperature the better is the appearance of the product. This is due to the fact that the little trace of Cutch that always remains on a large scale prevents the drying by filling the interspaces of crystals if not reduced to an extent when the crystal mud is porous and dries rapidly; even then the Cutch rises by capillarity and gives a dark red colour to the surface of the cake, the interior being pink. It is possible, however, to crrry the washing to a stage when a uniformly pink Katha is produced, but in that case a large amount of Catechin is dissolved and is lost in the washings to be added to the Cutch which has a lower market value. On the other hand it is always better and more profitable to keep a little Cutch in the Katha, provided it does not delay the drying, because being heavier it adds to the weight and increases the value of the Katha produced. Hence a standard machine-made Katha is drak reddish on the outside of the cake and pink inside. The crystals are long needles under the

microscope and are soluble in ether and other organic solvents. We need not examine the other properties of Cutch and Katha as they have no special bearing on the process of manufacture at present employed.

Ramnagar Factory.—The process adopted at the Ramnagar Factory was briefly as follows:—

The main features of the plant used were:—(1) a Disintegrator (2) a battery of cement tanks for extraction (3) an improvised still for vacuum-distillation (4) crystallising tanks (5) Filter-press and (6) a vacuum drying apparatus.

As stated above, the attempt was not profitable but the following important points came to notice:—

(1) The use of a disintegrator is neither efficient nor economical.

(2) The extraction is not uniform and not sufficiently rapid as the liquid and chips are practically in stationary contact. Thus the film of liquid immediately in contact with the chips becomes saturated and retards further extraction.

(3) There is a large waste of time and labour in digging out the exhausted chips.

(4) The bulk of the liquid extract and chips being considerable, a comparatively large space is required to treat sufficient wood required to give only a small product.

(5) The drying of Katha in the open is practicable only on a small scale and for a part of the year.

(6) To make the industry profitable on a large scale it is quite necessary that a sufficiently large quantity of wood must be

treated by a compact plant capable of carrying out the operations much more rapidly than in the case of the Ramnagar Factory. Otherwise the overhead charges kill any prospect of making a profit.

Indian Wood Products Co., Ltd.—The concern at Bareilly adopted a modified process based on this experience. The following is a description of the plant and further experiments in England. The wood in logs of about 3 to 4 feet in length was brought to the yard by a railway sliding to the place whence it could be directly supplied to the reducing machinery by men alone or by means of trollies on the side of the wagons if they are unloaded a bit apart for want of space. The Chipping machinery consisted of a pair of rotating chippers on the other surface of each of which 32 knives were fixed. The edges of these knives were alternately plane and serrated. The whole was encased in a protective sheath, the machine rotated with a high velocity and chipped the logs to the required size, by the forcible knife-strokes on the logs pressed against it by labourers. The two chipping machines worked alternately day and night cutting 40 tons of wood. The machine not at work was fitted with freshly sharpened knives and kept ready for its next turn. A sharpening machine always worked to keep up the supply of sharpened knives and a substantial excess in reserve.

The Elevator.—An elevator of the chain and bucket type came next to remove the chips as they came out and fell in the pit below, lifting them to a height of 40 feet where they were dropped on the wooden floor of a high building. A few labourers then shovelled them to closed openings in the floor so situated that when the door was released the chips fell directly in the man-holes of large copper autoclaves erected in the lower part of the building.

Extraction Plant.—The extraction battery consisted of five closed copper autoclaves with man-holes at top and bottom. Arrangements were made to keep the extracting liquid in constant circulation by pumping it out of the autoclaves at the bottom and dropping it on top of the chips by means of an external pump. The remaining fittings consisted of the usual type, outlet and inlet pipes for water, steam, air, extract, etc., the levels of liquid could be seen from guage-glasses fixed outside and the temperatures and pressures determined by thermometers and guages. The transmission of liquid from one autoclave to another or to storage tank was managed by a system of pumps, valves and pipes just as in the case of a fermentation battery; each autoclave had a capacity of 2,000 gallons. Four autoclaves were always engaged, the fifth being meanwhile filled with fresh chips and kept ready for extraction when a former one was finished and emptied. The extracted chips dropped down from the bottom man-hole on the ground floor to be thrown as required directly on the top of the specially constructed furnaces which burnt the wet chips and supplied the heat to the boilers, The liquid extract was pumped to a reservoir whence it was taken by the evaporating plant.

Evaporators.—The evaporating plant was divided into three sections designed by Messrs. Kestner & Co., the first two being of the vacuum film evaporator type and the third, film evaporator without vacuum. The first section called the "First Triple" consisted of three cylindrical parts called Callandria, erected vertically and in series. The cylinders contained a system of long tubes fixed on both sides in the holes of perforated plates which formed top and bottom respectively of two chambers at the bottom and top of the callandrium. A vertical partition divided the chamber into

two sections. At the top of each callandrium was a condensing chamber for vapours each of which was connected with the next callandrium while the the last one was connected with a vacuum pump and barometric column of water. In addition, pumps and pipes, for supply of condensing water, steam, liquid for evaporation, extraction of condensed vapour and outlets, traps, etc., were fitted as usual.

The second section of the evaporating plant was nearly the same but of a smaller size and with addition of a pre-heater as the feed came from cold crystallisation tanks instead of from the hot extraction battery as in the case of the first triple.

The third part called the "Finisher" consisted of only one callandrium of a similar construction with a pre-heater but without any arrangement for vacuum. This cylinder dropped the concentrated stuff in a separate bottom chamber before being ejected by a valve to flow slowly through an open wooden trough into the packing boxes at the other end. All these were fitted with the necessary guages, etc.. The plant was worked as follows:—The feed-liquid rose in a thin film on the inner surface of the several tubes connected with one part of the bottom chamber which received the feed. This thin film enclosed a column of its own vapour as an inner core and both rose at the top into the corresponding section of the top chamber where the vapour escaped and condensed to water to be drawn out by the extracting pumps. The liquid accumulated and overflowed the partition into the other section of the chamber to descend down the remaining tubes in the same fashion and dropped in the corresponding section of the bottom chamber. Thence it passed to the second and third callandria in succession and underwent the same treatment. The last callandrium of the first triple took the liquid through a refrigerating system to cold crystallisation

tanks made of masonry and concrete. In the case of the second triple the last callandrium dropped it into copper tanks which fed the finisher. The vacuum pumps maintained a vacuum of 25 inches or more over the concentrating liquids and other pumps extracted all condensed water. The first triple was designed to evaporate four hundred gallons of water per hour, the second three hundred gallons and the finisher a hundred gallons. The liquid from the first triple was concentrated to the required density necessary for successful crystallisation of catechin and sent to the concrete and masonry tanks of the cold room.

Cooling, Crystallisation and Filtration.—The refrigerating system consisted of two sets of cooling tubes the first cooled by the ordinary water while the second was cooled by refrigerated brine if necessary. The brine was stored in a masonry tank with arrangements to cool it by means of ammonia compressor of the usual type used for making ice. The cold room was a covered chamber closed on all sides. The liquid could be sent in or out by means of valves on the outside. Each tank was allowed a rest of 16 to 24 hours during which time all the Katha crystallised out making the liquid turbid and very thick. The thick liquid was then sent through filter presses where the Katha was cought between the cloths as a cake. The filtrate which was very much thinner passed out and along with the washing was sent to the second triple for further concentration and then on to the finisher for final evaporation. What came out of the finisher was pure cutch containing no Katha and ready for despatch after proper setting and packing.

Drying.—The mud on the filter cloths was shaped into irregular rectangular two-inch cubes and dried on wire gauze shelves placed in a drying room similar to that used for drying cordite at Aravankadu near Wellington on the Nilgiris and warmed by hot air heated

by the waste flue-gasses and driven inside the room by a fan. After complete drying the Katha was ready for sale. In addition to this there was the usual power plant driving all the principal machines direcly by belt-drives; no electric transmission of power was designed probably for financial reasons.

The Process at Bareilly.—The sizes of the various sections and the procedure were determined from data accumulated from Ramnagar experience and special additional experiments done in England. The process may be briefly described as follows:—Forty tons of logs with bark were chipped by the reducing machinary at the rate of two tons per hour allowing for stoppages and working 22 hours a day by two shifts. It would have been very costly to remove the bark and hence the whole log was chipped. Experiments had shown that complete extraction of the wood is effected by ten to twelve volumes of hot water at the atmospheric pressure for 15 minutes. It is immaterial whether the water is added at once or in instalments. It is an advantage to use boiling water at least for partly exhausted chips. The autoclaves were therefore fitted with two tons of chips and three volumes of water and the water kept in brisk circulation. The liquid was changed four times in the hour so that each lot of chips was extracted four times with three volums of water at a time making twelve volumes in all, fresh water always coming in contact with exhausted chips which were then taken out at the bottom for use as fuel. Thus the extraction battery gave out about 900 gallons of extract containing 7·5 per cent. total solids and having a gravity of 5·64° TW. Along with this four tons of exhausted chips containing more than 50 per cent. water were available for use in the furnace and on calculations based on their calorific value which is 9172·8 B. Th. units having an

evaporation value of 8·5 lbs. of water, they were found to be more than sufficient for the fuel requirements. As there was no sulphur in them they were a safe fuel and the existence of water in large quantities was no obstacle as it helped to give a sort of producer gas when burnt in specially constructed furnaces. Owing to evaporation losses in large scale work, the liquid that actually flowed out of the autoclaves was expected to be 8000 lbs. of 6·9° TW. containing 8·4 per cent. total solids, i. e., 6·70 lbs. Thus the first triple would remove 4000 lbs. extract of 15° TW. containing 20 per cent. solids or 670 lbs. and 3330 lbs. of water to pass to the cold room. The refrigertating plant and presses should have the same capacity to treat 4000 lbs. of liquid. After pressing, one-third of the total solids were removed as Katha leaving 446 lbs. cutch and 3330 lbs. of water and 500 lbs. of added wash water, a total of 3830 lbs. of liquid with 8·6° TW. or 11·65 per cent. solids. The second triple evaporated from this 3000 lbs. of water and left 830 lbs. containing 4046 lbs. of solids of this the finisher evaporated 100 lbs. and left 730 lbs. containing 446 lbs. of solids. There was a further loss of water as the Cutch flowed out in the open trough where it was also aided by hand stirring. The stuff that was ultimately collected in boxes was found in actual practice to contain nearly 20 per cent. moisture which lessened on standing to about 10 per cent. if the cutch was properly made. 170 cubic feet of chips weighed one ton and hence the autoclaves could easily take two tons packed by its own weight and yet leave sufficient margin for water, vapour, etc. There were three tanks in the cold chamber each with a crpacity of about 3,200 gallons. The lower the temperature in extraction, evaporation and drying, the lighter is the colour of the finished products. The temperature of the liquid in the cold room was maintained between 60° to 70° and the Katha was dried at about 100° F. The above

summarises the process as at first designed but on actual work several unexpected difficulties arose as follows:—

The old logs in stock were so hard that the machines could not chip the expected amount and the knives became very seriously damaged. The serrated edges had no advantage as expected but on the other hand the projecting teeth snapped off very easily thus enabling the plane knives to give a better result as regards cutting and wear. Fresh green logs could be chipped with ease but the chips and the extract fermented and created a nuisance. It was observed that within a few hours such chips on stocking became dark in colour and very hot, the temperature of the heap rising to 49°C. Frequently the machines and the lower crag wheel of the elevator got jammed with long pieces of wood or bark. This was more so with green logs and caused frequent stoppages. Pieces of wood flew around and struck the workers with such force that they were sometimes fatally injured. Thus the necessity of a screen in front and some protection for the faces of labourers was apparent. There was once a fire owing to the dust getting between the oiled bearing and other places. Though these were only mechanical difficulties they had immense effect on the output and for a time became the main problem.

The autoclaves generally worked well and provided sufficient chips were available there was no difficulty in maintaining the heat for boilers when once the workers had learnt how to charge the furnaces.

The evaporating plant gave some trouble in the beginning and sometimes the working became so fitful that instead of a uniform product, liquid of various densities came out of the finisher. It was then shown that the pressure of the superheated steam must be kept below such limits the liquid film on the inner surface of the several tubes should not be dried then

and there and insulate that part from further heating. Such a state would preclude uniform heating and some liquid wood escape concentration. The unequal heating of the tubes may wear them easily. This was borne out by the fact that an amount of charred substance was actually scraped out of tubes on one such occasion when after trying every other means the finisher was dismantled and cleaned. It was afterwards made a normal practice to wash the triples and finisher after each shift, whenever there was a break in the operation or when they began to work irregularly. It was also made a rule not to use high pressures for steam supply.

The liquid in the cold room fermented when green woold was extracted and the Cutch and Katha could not be satisfactorily separated by the filter presses. It was then shown that the fermentation should be allowed to complete to get anything like a good product otherwise logs stocked in the yard more than a year should be used. An additional space in the cold room would be required if green wood giving a fermentable extract is to be used. The chipping machines must be able to cut old wood in sufficient quantity if that is to be avoided. The presence of an undue proportion of gum, however, is a grest nuisance whether with regard to the manipulation of Katha or Cutch. It causes fermentation in the tanks. It makes the finished Cutch incapable of permanent setting however much the moisture in it is reduced. The Cutch softens and wherever it goes is the cause of considerable trouble. The Katha dries in the room much more quickly if free from considerable quantities of Cutch or gum especially if the moisture is considerably reduced by a previous absorption on sand-beds raised on brick-structures allowing complete ventilation at the bottom. Otherwise the fermentation of the gum raises like the dough paste on the sand-beds. In that

case no further reduction in moisture takes place, the mass being full of gass bubles. It is imposible to give proper shape to such material which has to be dried in lumps and when dried after a long time they are black, friable, foul-smelling, vesicled, irregular pieces which no body buys.

It is therefore extremely necessary to reduce the gum content of the extract. This can only be done by using old wood only or a mixture of old and green wood according to the capacity of the chipping plant. If the chipping of old logs is not sufficiently economical then sufficient space in the cold-room must be kept to stock the extract until the fermentation is complete. But in this case the Cutch from the finisher will be plastic unless it is further treated in drier or other arrangement. The Firm of Messers. Kestner & Co., have devised an additional attachment to their plant which is expected to dry the material still further and produce it in a form capable of being packed in ordinary bags.

The Cutch as it comes out of the finisher is a poorer dye than Burma made Cutch and it has now been conclusively shown that further *roasting* preferably in the open and at as high a temperature as possible increases the dyeing power which then goes up even higher than that of Burma made material. This fact clearly disposes of the argument sometimes raised that the lower tinctorial power is due to separation and removal of catechin. On the other hand pure catechin has been shown to possess no dyeing power. The open fire used to heat directly the iron pans while making Burma Cutch increased the active dye content while the vacuum evaporation at a comparatively low temperature does not favour that action. The contention that the iron in the Burma Cutch is responsible for greater tinctorial value has been disposed of by producing Cutch of high

dyeing power without using iron in any form. Thus the dyeing power of Cutch has no relation to the Catechin or iron in it but simply depends on the process of preparation.

This industry is obviously in its infancy and hence capable of further improvements. However, it is a very interesting development on modern lines of a crude industry suggesting the possibility of a similar development in other cases.

XXII

UTILISATION OF TANNERY WASTE

BY

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and

V. S. Chinnasami, B. A.

DURING the preliminary processes of treating the raw hide and converting it into pelt, pieces and cuttings of the swollen hide are obtained as by-products during the operation technically known as fleshings. The side on which the hair grows is called the grain side and is naturally smooth and even. The flesh side has however bits of dried up flesh and sinew sticking on, which during liming swells up and are removed by a sharp knife in such a way as to leave a fairly smooth under-surface of even feel. The pieces that are removed are known as fleshings and are dried in the sun and stored.

Previous to the war, these fleshings found their way into Germany; but when this became impossible, the fleshings accumulated in such large quantities as to become a nuisance to the tanners. It was generally known that glue and gelatine could be made out of it. But the actual process of manufacture were kept so secret in Europe and the continent, that it was not realised how simple they were. The fleshings are first cleared of lime and dirt, boiled with water and the resulting soup is, after concentration, allowed to cool. If the temperature is not above 78° F. the concentrated solution sets to a solid which could be cut and dried at a low temperature. The resulting product is glue. During these operations the soup must be kept free

from spores, bacteria and enzymes to prevent these from bringing about decomposition and ultimate putrefaction.

There are thus three difficulties at least which the glue manufacturer in India has to fight against; these are (1) liquifying organisms, (2) low temperature jellyfying and (3) low temperature drying. Rideal and Lambert, the only authorities on this subject are certain of the impossibility of making glue solutions act at temperatures higher than 78° F and the scanty literature that is available on the subject is sufficient to indicate the futility of the manufacture of glue in the tropics.

It was recognised early during the course of our work that European methods of manufacture involving the maintenance of low temperature are utterly unsuitable for Indian conditions. The first problem therefore, was to make glue solutions set at higher temperatures, and it may be interesting to detail here the manner in which this was accomplished.

Put briefly, the hide substance is made up of carbon, hydrogen, oxygen, and nitrogen atoms, linked together in a closed system. Trypsin—an active enzyme capable of attacking open chain compounds—is unable to attack the Collagen of the hide and proves to a certain extent the existence of ring structure. It is well-known that on digesting Collagen with water, hydrolysis takes place, the resulting substance being gelatine, an open chain amphoteric organic complex which can be split up into various nitrogenous degradation products by the enzyme trypsin. This hydrolysis is progressive and if carried to a finish yields various proteoses, peptones and peptoses as well as leucine, aspartic and other amino-acids. The first product of the gentle hydrolysis of Collogen is the protein gelatine; and glue has been shown to be gelatine with a small proportion of its own disintegration products.

The first series of experiments was therefore directed towards the determination of the optimum conditions, both chemical and bio-chemical, under which the first process of the digestion of fleshings should take place. This having been ascertained it was necessary to convert a dilute solution of gelatine into solid gelatine, or glue. The question is purely then one of the removal of water, but is complicated by the fact that as evaporation of water goes on, progressive hydrolysis of the gelatine is also going on. Ordinary methods of laboratory evaporation in air or *vacuo* yield in Madras a substance which is highly hygroscopic and is decidedly not glue. Under European climatic conditions the dilute soup obtained on digestion sets immediately on cooling to a solid which on drying loses its water without simultaneous hydrolysis of the gelatine molecule and finally yields a definite product containing no more than about 15 per cent. of moisture.

It requires no great intelligence to see that the actual problem depended for its solution on the result of a race between the progress of concentration of the solution and that of the decomposition of the gelatine molecule and our work was directed towards imposing as great a handicap as possible on the latter. It is not necessary to detail fully the experiments that were directed towards the line of our advances.

Melting points of gelatine jellies were recognised to be a linear function of their gelatine contents. Thus a more concentrated solution of gelatine sets to a solid at a higher temperature than a less concentrated solution. But this relation holds good only within certain limits. Thus a 1 per cent. solution of good gelatine sets at about 11° C. and a 25 per cent. solution of the same gelatine sets at about 28° C. while even a 50 per cent. solution does not set at 35° C. This showed that even if considerable concentration of the solution

were effected in a manner that would prevent the decomposition of the gelatine molecule the liquor obtained would not set at the maximum Madras day temperature. At this stage a lucky idea opened the way for a new train of thought which, as we shall show presently, led to the ultimate success.

Photographers are familiar with the fact that the sensitive gelatine film is destroyed on washing with warm or even tepid water. A common contrivance to get over the frilling of the gelatine, as it is technically known, is to harden it by immersion in a dilute solution of alum after which the plate may be washed in fairly warm water. Here was the phenomenon of the melting point of gelatine being profoundly affected by the application of a chemical and this naturally demanded immediate investigation.

Very careful work done in this direction revealed a very interesting state of affairs. Potassium aluminium sulphate added in a certain proportion dissolved in dilute gelatine solutions; on boiling however it entered into some type of combination, yielding an insoluble product which settled down clarifying the gelatine solution. Added in certain other proportions the salt completely precipitated the gelatine from solution and this precipitate could not be reconverted into gelatine. This pointed to the existence of an optimum concentration at which Potassium Aluminium Sulphate would have the same effect on a gelatine solution as on a gelatine film without however any precipitation taking place. This point was ultimately discovered and the method of setting glue solutions at tropical temperatures duly covered by a patent. This method of setting gelatine solutions at high temperatures could not lead immediately to the creation of a new industry. Practical issues of a difficult nature arose in translating what was a laboratory practice into a large scale one. The question of

antiseptics had to be studied from the bio-chemical and bacteriological standpoints. The efficiency of different evaporators had then to be gone into which led to the designing of suitable models. Lastly came up the question of economics, competitive prices and finish of the product. Most of the difficulties have been tackled with some success. An important investigation now in hand relates to the enzymatic digestion of fleshings. Certain bacteria obtained from putrifying hide would have been found to secrete enzymes that could delime the wet fleshings and at the same time dissolve out the mucilaginous organic matter that binds the collagen together. Experiments carried on with these bacteria show that the resulting tryptic digestion makes the hydrolytic digestion easier and thus cuts down the cost of production.

XXIII

PHYSICO-CHEMICAL CONTROL IN THE MANUFACTURE OF GELATINE AND GLUE.

BY

R. Nilakanta Warriar, B. A.

ALTHOUGH gelatine and glue are commercially different substances and find quite different application in industries and arts, they are very much allied in chemical constitution. In standard technological works on the subject, one often meets with the statement that gelatine is only a highly purified form of glue; but a careful study of the chemical and physical properties of both the substances cannot be said to confirm this view. For gelatine in its pure form is a hydrate of collagen, whilst glue, besides proteins other than collagen, contains an appreciable amount of the disintegration products of protein *viz.*, proteoses peptones and amino-acids. In fact, when a solution of gelatine is heated for a few hours, it gradually loses its jellying power and acquires correspondingly greater adhesive strength until a certain stage, thus approximating to the condition of glue. Jelly-strength and comparative freedom from the decomposition products of protein and other extraneous matters, are the characteristics of a good gelatine, whilst in the case of glue, adhesive strength is the chief characteristic, although in comparing different grades of glue, jelly strength is a good indication of quality. Both glue and gelatine are made from animal bye-products by identical processes, the selection of the raw material and the details of the operations being altered to

suit the requirements of the finished product. The making of edible gelatine requires careful selection of the raw material and more vigilant control over the various operations than the making of ordinary gelatine, whilst the manufacture of gelatine in any form requires greater care than the manufacture of glue.

Animal tissues such as fleshings from hides and skins, connective tissue, bones, cartilage &c. form the raw material from which gelatine and glue are made. The proteins present in the bodies of animals all come under the class of sclero-proteins, but proteins in different parts of the animal body have slightly different characteristics.

The following scheme taken from Dr. Bogue's "Chemistry and Technology of gelatine and glue" gives an idea of the nature of the poteins present in various parts of the animal body :—

Raw Material.	Nature of Protein Present.
Hides and Skins.	Mainly Keratin and Collagen and Elastin.
Sinews and tendons.	Collagen and Elastin with small quantities of Mucoid &c.
Cartilage.	Mainly Collagen, small quantities mucoid and chondrin.
Bones.	Collagen and small quantities of mucoid, chondrin &c.

According to Hofmeister, Collagen has the empirical formula $C_{102}H_{149}O_{38}N_{31}$ and when it is treated with water at 90°—100° C. it combines with one molecule of water giving gelatine. It will be evid ent from the scheme given above that in the manufacture of gelatine on a commercial scale, it is not possible to restrict the raw materials to collagen containing substances only, for there is no part of the animal body in which collagen is present unassociated with some one or more of the other proteins

keratin, chondrin, mucin &c. gelatine or glue as obtained in commerce cannot therefore be said to be a compound of definite and invariable chemical composition. We can however, by intelligent selection of the raw material and by careful chemical control of the various manufacturing operations, obtain a product which approaches pure gelatine or glue in constitution and properties.

In ancient days glue and gelatine were made by empirical methods, much in the same way as cooks prepare curries without any idea of the chemistry of the raw materials used in the culinary art. It is only recently that attention has been given to the chemistry of glue and gelatine. The recent developments of protein chemistry and the study of their colloidal behaviour have thrown much light on the whole subject, and have enabled chemists to alter the the time-honoured manufacturing methods, so as to yield really high-grade and standard products. It will therefore be evident that if the manufacture should be a success, the strictest attention should be paid to the smallest trifles and very careful chemical control exercised over all the operations. In the following paragraphs, a brief description is given of the various processes in the manufacture of gelatine and glue and the more important points will be indicated where strictest control is required.

When hide-pieces, fleshings from the tanneries etc., form the raw material, they are first thoroughly washed in specially devised machines—usually paddle-washers, or preferably drum-washers—whereby much of the coarse extraneous matter such as blood, dirt, grit, adhering lime in the case of fleshing etc., are removed. The next operation is liming. If, however, the material has been subjected to a liming operation before, as is the case when hide fleshings from the tanneries are used, this second liming is not required;

but when raw hide-pieces from the slaughter-house are used, it is essential that they should be limed. The objects of liming are (i) to dissolve away the blood and epidermis of the skin; (ii) to saponify the fatty matter, which is thereby converted into insoluble lime soaps ; (iii) to preserve the skin from bacterial action if it is not immediately used and (iv) to soften the stock; liming also appears to effect a transformation in the tissues—the mechanism of which is not clearly known—whereby the glue-yielding substances are rendered easily extractable in the subsequent digestion. The operation of liming is conducted in wooden vats, and the material is steeped in the lime solution for a few days, which it is kept constantly stirred by wooden rakes. The period after which the material is to be removed from the lime-pits differs according to the nature of the raw material and temperature conditions, and should be determined by experience alone. The limed hide-pieces can be dried in the sun and kept for future use if necessary; if used immediately they are washed thoroughly from all traces of adhering lime.

The raw material which has thus been prepared will still contain some free lime absorbed from the steeping tanks. If subjected to digestion in this state, this free lime attacks the glutinous matter and vitiates the product; in fact, a considerable proportion of the valuable collagen will be hydrolysed if digestion proceeds in the presence of caustic lime. It is therefore of the greatest importance that the alkalinity of the stock should be neutralised with suitable reagents, before the material is subjected to the process of extraction. There are various methods in vogue for effecient de-liming, and those which are the best in the writer's opinion will be briefly considered here.

1. The stock is steeped in water in a tank, and neutralised with dilute $H_2 So_4$ the exact quantity

required being calculated by testing a sample ; the calcium sulphate thus formed is precipitated in the course of digestion, and does not interfere with the glue liquor.

2. De-liming can be effected by passing carbon dioxide into the steep-water, calcium carbonate being precipitated in the course of digestion.

3. A small quantity of the enzyme trypsin is added to the steep-water with which the material remains in contact overnight. The action of this enzyme is said to be selective, attacking elastin and and leaving collagen. The amino-acids formed in the enzymatic hydrolysis of elastin form insoluble salts with the lime. It has been observed, however, that prolonged contact with enzyme appreciably affects the the collagen, and it has been the writer's experience that unless great care is exercised in adjusting the period of contact with the ferment, the whole stock begins to deteriorate in a few hours. The method, though simple, is difficult to control, and cannot be said to possess any advantage over the preceeding ones. Whatever be the method employed, it is essential that the digestion of the stock should take place in a neutral or slightly acid medium. An acidity represented by Ph=4.78 is said to give the best results. In fact, most of the failures in attempts to manufacture glue or gelatine, can be traced to the neglect of this most important factor.

After de-liming, the glue-stock is softened if necessary by steeping in water in cement tanks. In all these operations, it is very important that the water used should be pure and should have a low mineral content. In the manufacture of gelatine distilled water should be used in the final washings and digestion. The period for which the material is soaked is a matter of experience only ; but normally a day's soaking is found to make it "plump" to the desired extent. It should be remembered that, if kept

for a longer time in contact with water, bacterial action will be set up and the stock will go bad unless protected by suitable antiseptics, sulphur dioxide being considered the best.

The next operation is 'extraction' or boiling the glue stock. The thoroughly washed and neutral material is put into the digester, which is a rectangular or cylindrical, iron or wooden tank, open at the top. The tank, if made of iron, may be steam-jacketed, in which case, a certain amount of water is poured into the digester, just enough to cover the stock, and boiled for a few hours until extraction is complete; but the best methed of extraction is to pass open steam into the material at a pressure of 25-35 lbs. The most important point to be noted here is that the quality of the finished product, whether glue or gelatine, depends to a very great extent on the time and temperature of digestion. As mentioned before, gelatine tends to break up into proteoses, peptones and amino-acids, if its solution is exposed to high temperatures for a long time. If, therefore the extraction takes a long time, the glutinous extracts as they are formed, are made to stew in the digester until the whole operation is finished, or until the extracts are removed. It is therefore advisable to remove the extracts from the digester periodically. For this, it is only necessary to provide the digester with a perforated false bottom, and a hole with a stop-cock below it to draw off the liquor. Thc second point to be noted is that the liquors obtained should not be too dilute, as in this case, the duty on the evaporators becomes heavy. For this reason, the method of digesting the stock by passing open steam is to be preferred,—The completion of extraction is easily recognised by examining a small sample.

After extraction, the glue or gelatine liquors are clarified. The reaction of the liquor is noted at this

stage, and if the previous instructions have been carefully followed it should be neutral to litmus or slightly pink to phenolphthalin. If it is alkaline, the quantity of Sulphuric acid required to neutralise is added. If the liquor is allowed to settle in this state, it will become clear in a few hours, provided the previous operations have been rightly performed; the clear liquor can then be pumped or siphoned from the top and does not require any further clarification. If however the liquor is not so clear, a small quantity of alum should be added in the from of solution, and after well mixing, the liquor should be heated to about 90°C. It should then be allowed to settle for an hour or two, and the clear liquor may then be pumped off for the next operation.

The clarified liquor is sometimes bleached, when a pale variety of glue or transparent gelatine is required. The bleaching is best done with sulphur dioxide gas. There are various types of sulphur burners on the market, and any of them may be used with success. The right quantity of sulphur to be burned depends upon the exact colour that is required and may be correspondingly adjusted. Sodium hydrosulphite is also used for bleaching with equal efficiency.

The next stage in the manufacture is evaporation of the liquor. Evaporation in open pans is out of the question, due to reasons given before, and it is absolutely necessary to make use of the latest machinery for the purpose, in order to get a really good product. Evaporation is generally conducted in two stages. The dilute glue or gelatine liquor, is first, concentrated in film evaporators—of which there are many well-known types—and afterwards finished by evaporating in the vacuum-pan. In these processes it is always necessary to remember that time during which the liquor is exposed to high temperatures should be as short as practicable. In the film evaporator of the Kestner type the liquor is in contact with

the heating surface only for a few seconds and in the further concentration in the vacuum-pan, the working temperature is much lower than 100°C. The liquor is drawn from the vacuum-pan at a concentration of 70—75 per cent. solids and poured on shallow trays made of galvanised iron or glass when it sets to a jelly.

Relying on the conclusions arrived at by some former workers, who were deputed by Government with the task of investigating the possibility of glue-manufacture in India, statements have often been made in the Indian press from which they have been copied by foreign scientific journals,* to the effect that glue or gelatine liquors will not set or will set only with difficulty by the introduction of novel processes in the temperature conditions existing in this country. This is far from the truth, and the statement therefore requires revision in the light of results obtained in the laboratory as well as in the factory. A high temperature does not interfere with the setting of a glue liquor, provided it is prepared under the right conditions, and is drawn from the vacuum-pan at a fairly high concentration.

The next and last operation is that of drying the glue cakes. This is effected in specially constructed drying chambers or tunnels, in which a continuous current of air is set up by one or more powerful fans revolving at high speed on one side of the room. A steam heater placed in front of the fan in the room raises the temperature of the incoming draught of air to about 38—40°C. and the air after traversing the length of the room escapes through holes made in the wall on the other side. Except for these holes, and the opening for the fans, the room is closed up all around and in the top. The glue cakes, after they are cut into the required sizes, are transferred to wire racks in the drying room.

*(J. S. C. I. Feb. 16—1923.)

At the temperature of the drying room bacterial contamination cannot take place in the course of drying. If the afore-mentioned details are carefully attended to in the previous operations, the subsequent drying becomes quite an easy matter, because the jelly formed will have sufficient gelatine content to stand a temperature of 38-40°C. without melting. Too much emphasis cannot be laid on this point as, recently, statements have been made in some articles on the subject that the drying of glue cakes is a difficult problem in tropical countries like India, where the daily temperature is fairly high. From the writer's experience of actual factory conditions in Madras, where the average daily temperature goes much above 100°F it can be said that drying presents no problem at all to the glue-maker in this country.

When bones are treated for the manufacture of gelatine or glue, the preliminary treatment assumes a different form. Bones consist of fat, mineral matter, such as calcium phosphate, magnesium phosphate and calcium carbonate, and organic matter ossein which is mainly composed of collagen. The aim of the glue-maker is to get at the collagen, without the fat or mineral matter appreciably interfering in the process.

The bones are first thoroughly sorted and freed from foreign matter such as stones, nails, rags, hair &c., and they are then passed on to the crushing mill in which a number of steel knives fixed to a shaft revolve in a chamber. In this machine, the bones are comminuted into small pieces.

In order to extract the glue-yielding substance from bones, it is necessary to remove the mineral matter by treatment with dilute HCl. They are then thoroughly washed and passed on to the digester without delay excepting that for the production of a high-grade gelatine it is preferable to remove the fat before subjecting it to the boiling operation. The fat is

generally removed by extraction with benzene in a solvent extractor. The details of the degreasing plant and the method of working cannot here be dealt with in detail; but it is necessary to ensure that all the fat is removed before the material is passed on for extraction.

The bones from which only the mineral constituents have been removed may be steamed in an autoclave under a pressure of 40—50 lbs., in which case the major portion of the fat rises to the top, the ossein dissolving in water to form the glue liquor. The fat may be skimmed off, but it is not possible completely to remove the grease in this manner, and the glue liquor will therefore contain a certain amount of fat imbedded in it. For this reason and because the liquor is exposed to a fairly high temperature in the boiling, the glue that is produced in this manner is of low quality. If on the other hand both the mineral constituents and the fat have been previously removed by processes indicated above, the ossein gets easily dissolved at a temperature of 90—100°C, and gelatine of good quality can thus be obtained. The remaining operations,—viz., clarifying, evaporating and drying, are conducted as in the case of hide-piece.

In the above paragraphs, an attempt is made to point out the more important factors in the manufacture of gelatine and glue. The manufacture of these articles is essentially a chemical industry, where the chemist must work hand-in-hand with the Chemical Engineer. India offers enormous facilities for the development of this industry; for, in her live stock resources, India stands first among the countries of the world, and the bye-products of livestock form the raw material for the manufacture of gelatine and glue. At one time, it was thought that the manufacture of these substances without costly refrigerators was almost impossible under the climatic conditions of this country, and intending investors have been scared away by the idea; but it is the writer's considered opinion that glue or gelatine of excellent quality can be made in India with the help of the machinery described above.

XXIV

ROLE OF ENZYMES IN THE CHEMISTRY OF LEATHER MANUFACTURE

BY

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ONE of the most striking characteristics of enzymic action is the ease with which chemical changes are brought about in highly stable bodies. To effect the same changes in the laboratory powerful reagents are required to be used. A preparation of lactase was found by Frankland Armstrong to hydrolyse one quarter of the milk sugar in a five per cent. solution in one hour at 35°C. whereas 2 N. Hydrochloric acid required at the same temperature about five weeks to effect the same amount of hydrolysis. Enzymes are catalysts produced by living organisms and according to the limitation put by Armstrong, they are selective colloidal catalysts present in living cells and destroyed by heat. The living enzymes colloidal in nature possess the physical and chemical properties associated with organic colloids.

It is necessary to consider in brief the structure and constitution of the hides and skins, in order to understand fully the rôle or the digestive action of the enzymes in the chemistry of leather manufacture. To the naked eye the skins of the various species of mammals appear entirely different from one another. On closer examination it will be found that the skins of the higher animals resemble each other and also those of lower animals, e. g. lizards, all possess the same general characteristics with substantial modifications in the epidermal structure and the arrangement of fibre bundles; thus a description of one species will apply almost equally to another.

The skin of all higher animals is automatically divided into two layers.

1. An outer layer or epidermis (epithelium.)
2. An inner layer or corium (true skin.)

These too layers are totally different in function and origin. From the epidermis originate and grow all horny structures like nails, claws, horns, hoofs hair sudoriferous and sebaceous glands, etc., and with slight modification of inorganic salts are all chemically identical with epidermis. The latter consists of living cells and during their growth and multiplication, the old ones, become further and further removed from their food supply, become flat and thin and finally die of starvation. The young cells are in the inner half of the epidermis, and adult cells in the outer half, the older position being known as epidermis and the younger as "*rete malpighi*". Between the epidermis and corium there is an exceedingly thin glassy layer known as "hyaline."

The corium or true skin is chiefly composed of connective tissue fibres. These are interlacing bundles of white fibres composed of fibrils of extreme fineness cemented together by substances somewhat more softer than the fibres themselves. The fibre bundles loosely woven in the centre of the corium and fat cells are formed in the interstices. This is specially observeable in sheep skins. Besides the connective tissue fibres, the corium contains a considerable number of fine yellow elastic fibres and also blood and lymph. Below the corium we have the "*panniculus adiposus*" which is the connecting tissue uniting the veins and body.

The skin is chiefly built up of albuminoid. The epidermis mainly consists of keratins, while the corium is largely collagen or the glue yielding substance. Elastin is found in the yellow fibres and some of the blood-vessels of corium. The walls of blood and lymph vessels are keratinous, while the vessels themselves contain soluble albumins and globulins. The

constitution of the hyaline layer is unknown and exceptional.

Pure keratin of epidermis is quite insoluble in water and dilute acids; digestive ferments render it soluble after some time and with great difficulty. In the process of leather manufacture the whole epidermis up to the hyaline layer has to be removed before tanning. *Rete Malpighi* is built up of young keratin while the outer layer consists of adult or dying keratin. Young keratin is more readily soluble that adult keratin.

Whatever may be the constitution, ***hyaline*** layer is extremely tough and resistant, practically unaffected by acids, alkalis, and digestive ferments, and is capable of taking a high polish.

The white fibres of the skin are either identical with gelatine or only differ from it in their molecular condition or degree of hydration. One important physical difference is that pure gelatin exhibits no fibres. Though they cannot be considered identical with gelatine, the ease with which they yield gelatine proves that the latter is the first derivative of the former. The fibres are composed of a substance allied to gelatine which has received the name of collagen.

The question of the substance which cements the fibrils of the connective tissue is one of great importance and has been studied by Reimer. Lime and baryta solutions split up the fibres of ***corium*** and forming smaller and smaller fibrils at the same time dissolving the cementing substance. The substance is thrown down as a floculent precipitate on neutralisation with acetic acid and is known as ***corïn.***

Yellow elastic fibres of the skin rival keratin in stability and insolubility. They are now known to be distinct chemically from gelatin or keratin and consist of the albuminoid, elastin.

Thus in brief is the chemistry of the skin, and the digestive ferments of the bating and puering processes of leather manufacture, which attack and bring about changes in the raw material to make it fit for tanning purposes.

BATING, PUERING AND DRENCHING.

The skins as is well known are generally limed for depilation and when this process in the manufacture of leather is complete, removal of lime is necessary. If deliming alone was desired this could have been done by acids, but the skins are to be brought in a soft and flaccid condition, with no alkaline swelling, and certain constituents of the skins which are not required in the finished leather must be removed. All the older processes did this by means of bacterial fermentation, but it is now recognised that it was by the agency of enzymes or digestive ferments which the bacteria produced; and several modern bating preparations utilise the tryptic or pancreatic enzymes derived direct from animals.

These tryptic and pancreatic enzymes are the subject of this paper and before dealing with them at length, we might study their action on proteids, gelatine, (dehydrated collagen or skin) etc.

They are very selective in their action—the tryptic enzymes attack epidermis matter and emulsify fats, but are unable to attack the hide fibre itself, while pepsin digests the latter but does not attack the epidermis or fats. Most natural enzymes are mixtures and it is quite possible that if we could separate them, we might find that each single enzyme could only digest one species of proteid; and if we know exactly what we wished to remove from skins, we might select the enzymes for that purpose. All enzymes, especially trypsin and pepsin are very sensitive to the exact degree of acidity or alkalinity of the solution in which they work, and each has an optimum hydrion

concentration; thus pepsins will act in faintly acid solution while the trypsins require alkaline media.

No adequate explanation has yet been given as regards the "pulling down" effect of these fermentative processes but it is plausible that they bring the alkalinity or acidity of the solution towards the isoelectric point of collagen Ph. 4·7 or N/50,000 which is the point of minimum swelling. The usual reaction of the bate is Ph. 7·0 and the optimum concentration for trypsin at 37° C. is Ph. 9·7.

As the proteids are broken down into amino-acids by these enzymes, their capacity for combining with alkalies or acids is increased by the added number of free carboxyls, and as the amino-acids themselves are very weak, either as bases or acids, they must ultimately bring down the hydrion concentration of the solution to a point very near neutrality.

Bating:—consists in steeping the skins in weak fermenting infusions of pigeon or hen-dung for a few days.

Puering:—is a very similar process in which dog-dung is substituted for that of birds and as the mixture is used warm, the process is generally complete in a few hours.

Drenching liquor:—is made by infusing bran in hot water and allowing it to ferment under the influence of special bacteria which produce lactic and acetic acids. The working of drench is comparatively simple in theory and depends on the production of organic acids which neutralise the lime of the skin. The drench ferments are incapable of attacking or injuring the hide and when the skin is attacked it is due to putrefactive and gelatinising organisms that find their way to drenches in hot sultry weather.

Bating and Puering though differing practically in many ways, are identical in theory and what follows

applies to both of them. The action is much more complex than that of drench, involving both chemical reactions and those of organised and unorganised ferments, and it is a matter of no little difficulty to say what proportion of the observed effect should be ascribed to each of these agencies.

Taking drenching first out of the three processes mentioned above—although it is employed as a means of cleansing and slightly plumping the skins after the lime has been removed by puering or bating, in calf-kid for instance, it is substituted for the use of bates. In the fermentation of bran the most important of the active ferments are two species of bacteria named by Wood *Bacterium furfuris* A & B which are very similar in form and action but produce somewhat better fermentation together than separately. Neither species has any direct action on the hide substance but ferments the glucose produced by the action of the Cerealin of the bran or the starch. A considerable quantity of hydrogen, carbon dioxide, nitrogen and small quantity of hydrogen sulphide are produced together with lactic, acetic, traces of formic and butyric acids and amines. An experimental drench gave the following on analysis :—Formic acid, 0·0306 gm.; acetic acid 0·2402 gm.; butyric acid 0·0134 gm.; lactic acid 0.7907 gm. per litre.

Formerly all the effect was attributed to organic salts of ammonia and its homologues and to amino-acids, phosphoric acid etc. It is now recognised that the effect of these chemicals is unimportant compared with the products of bacterial action, and the researches of Wood have cleared up much that was until recently inexplicable.

Much effect is ascribed to the digestive ferments such as pepsin and trypsin which are present in fresh dung. It is known that the animal organism secretes these in considerable excess of its requirements.

Pepsin is the active principle of the secretion of the glands of the stomach and large quantities are prepared for medical use as an aid to digestion from the stomachs of pigs. Pepsin only acts in slightly acid solution and though fresh bate liquor is slightly acid to litmus it speedily becomes alkaline from the lime of the skins and the ammonia present so that the action of pepsin in a bate can only be a very limited one. Wood compared the action of a 1 per cent. solution of pepsin acidified with 0·2 per cent. hydrochloric acid, with that of a dog's dung puer liquor both at the temperature of 40° C. At the end of an hour the skin in the pepsin solution was considerably fallen but that in the puer solution was almost dissolved. Since the solution employed was much stronger than is likely to occur in practice and the conditions much more favourable to its action, it may be assumed that the practical effects in the bate are negligible. The peptic enzyme of the larger intestine which are likely to occur in the dung are bacterial in their origin and though 0·2 per cent. hydrochloric acid is the optimium for the pepsin of the stomach it is too high for those of the intestines.

Trypsin or Pancreatin is active in neutral and in alkaline solutions. It is the product of pancreas and is largely concerned in intestinal digestion. Chemically it much resembles pepsin but is more resistant to heat, retaining its power of digestion after heating to a temperature of 160°C. in a dry condition. Its warm solution dissolves fibrin almost instantly and in large quantity and peptonises gelatine so as to render it soluble in water. Wood found that a 1 per cent. solution of trypsin acts far more rapidly than a solution of pepsin of equal strength. At 40°C. the skin fell rapidly and the action continued in the cold. In fifteen hours the solution was swarming with bacteria and on repeating the experiment with the addition of 15 per cent. chloroform prevented the development of

bacteria while the trypsin continued active. The skin fell as before but in neither case had it the peculiar touch of puered skin. Wood found later that it was due to the presence of an excess of lime and the want of ammonia which is necessary to activate the pure pancreatic ferment. In the body this is done by a special enzyme *Enterokinase* which is secreted in the small intestine. The practical pancreatic bates such as "pancreol" and "orpon" contain ammonium chloride.

A fresh puer-liquor when boiled for half-an-hour and so freed from living organisms and albuminoids has still considerable effect on limed skins though much less than unboiled puer. Wood found that the action was principally due to amines, and their compounds with organic acids which removed lime but did not remove the interfibrillary substance or give the proper feel of the bated skin. One per cent. aniline hydrochloride gave very similar results.

A considerable variety of bacteria from dung were cultivated and their puering power tested but though greater than that of amine salts etc., it was not equal to that of an ordinary puer. When, however, a small quantity of the amine salts obtained from the puer was added to a mixed bacterial culture, the effect on the skin was almost as rapid and considerable as with an actual puer.

In order to determine whether the puering effect was due to the direct action of the bacteria or to their enzyme products, the latter was separated by adding it to a large volume of 98 per cent. alcohol in which enzymes are insoluble. On redissolving in water they had a decided puering effect and a solution of 0·5 gm. of the mixed enzymes and 0·5 gm. of the mixed amine chlorides in 100 cc. of water at 35°C. brought down a piece of limed skin in 30 minutes exactly like a puer. The action is therefore dependent on the mutual action of the enzymes and amine salts.

In addition to trypsin, the pancreas also secretes the enzyme steapsin which emulsifies and saponifies fat, but like trypsin is inert as secreted and requires to be energised by ammonia or enterokinase.

Considerable light has been thrown on the real object of puering, which obviously accomplishes more than the mere removal of lime and of the remains of the hair follicles and fat glands, by the work of Rosenthal, Wilson, Moellor and Seymour-Jones who have pointed out that its most important object is the digestion and the removal of the elastin fibres which are very abundant in the grain layer and prevent its stretching.

Mechanism of Bating.—The object of bating is to prepare the unhaired skins for tannins so that the plumpness disappears and the skins become so soft as to retain the impression of thumb and finger when pinched and sufficiently porous to permit the passage of air under slight pressure.

Our knowledge of the behaviour of proteins in contact with aqueous solutions of acids, bases and salts, in which protein swells by absorption of water to an extent depending upon the nature and concentration of the electrolyte, raises the question as to whether bating is not simply a means of bringing the skins into a condition of minimum swelling especially since such a condition would give the skins those physical properties which are widely accepted as indicative of properly bated skins.

An experiment was tried by taking 300 cc. graduated cylinders and in each 2 gms. of standard hide powder were placed. First cylinder was filled with saturated lime water, the second with distilled water and the third with a bate liquor showing a value for log. H of 8·1. The cylinders were stoppered and shaken at intervals and the swollen powders allowed to settle. At the end of 8 hours the volume occupied by the powders were as follows;

in lime water 41 cc. in distilled water 32 cc. and in the bate liquor 31 cc. showing that bate liquor actually causes less swelling of hide powder than distilled water. A pure solution of NH_4Cl of the same concentration and alkalinity as the bate liquor produces practically the same degree of swelling. Another test made by comparing the action of ammonium chloride alone with that of commercial bate containing pancreatin and ammonium chloride. Both were made up to the concentration of 1·20 gm. of ammonium chloride per litre and skins of similar nature were put into each and all other conditions kept alike. At the end of several hours, the skins in both liquors had the properties of bated skins and no difference could be detected. This test indicates one of the two things—either that pancreatin was of no practical benefit or else that the commercial bate was deficient in enzymes.

Some years ago, Rosenthal concluded that the bating process removes elastin from the skins. In a sample from the butt of a calf-skin he found 10·36 per cent. of elastin before bating and only 0·31 per cent. after bating. He could not prove it conclusively; what he proved was that bating removed almost to completion certain nitrogenous matter from the limed skin.

The statements of Moeller in Germany and Seymour—Jones and Wood in England that elastin is removed by bating was proved by an interesting experiment. The "flywing" grain of sheep skin was split from the main body of the skin called flesh for convenience and both grain and flesh were cut into halves along the backbone. One grain and one flesh were bated with pancreatin while the other halves were de-limed with acetic acid but not bated. All the four pieces were then tanned with sumac.

There was comparatively little difference between the bated and unbated flesh-halves but the grain samples

were very different from each other. The bated grain was soft and even, with the hair holes clean and clear, but in the unbated grain the hair holes appeared to be glued up and the surface had a rough contracted appearance. Seymour-Jones concluded that elastin is present only in the grain membrane and that it must be digested before tanning to produce a satisfactory grain membrane but that bating of the skin under the grain is not only unnecessary but often undesirable.

It was further decided to settle definitely the question of the removal of elastin in the bating process by means of photo-micrographs of cross sections of the skin taken before and after bating. The elastin fibres are not clearly discernible unless stained with magenta which makes them more prominent by darkening them in colour. Two liquors were prepared each containing 1·20 gm. per litre ammonium chloride while one also contained 0·003 gms. per litre of U. S. P. grade of trypsin. Hydrion concentration was brought up to log. H-8·0. A limed piece of calf-skin was kept in each for 24 hours at 37°C. Microscopic examination of the sample from the trypsin liquor showed that practically all of the elastin had been removed while, in the piece treated with ammonium chloride, the elastin was left unaltered. The test on a large scale gave the same results. The time factor in the digestion of elastin can be followed by examining the sections at intervals.

These experiments fully confirm Seymour-Jones' observations that elastin is digested by trypsin. Besides this tryptic activity in bating it has been found that pepsin easily attacks the ordinary connective tissue fibres in the presence of of hydrochloric acid, but trypsin does not. Connective tissue may, therefore, be obtained in the pure state by removing the other albuminous bodies present

with trypsin. Some investigators view the bating process as one by which the cementing substances of the skin are removed and they do not consider the removal of elastin fibres as an essential feature of bating.

In a most recent paper by Stiasny and W. Ackermann (ueber die Wirkung von Trypsin auf kollagen und die Beein flussing dieser Wirkung durch Neutrasatze Collegium 1923 Oct.) they have conclusively proved that (1) the effect of trypsin on Collagen depends essentially on its preliminary condition notably on the degree and its swelling, (2) increase in the quantity of trypsin of raising of temperature both increase the tryptic activity on Collagen without modifying the relation that exists between swelling and proteolytic action. (3) swelling of skin and proteolytic action of salts generally go in pairs but the action of ferments, predominates (4) Peptisation of skins takes place in all concentrations of trypsin.

The extent of our present knowledge of the action of enzymes in puering may be summed up as follows:— Active enzymes are produced by bacteria growing in an infusion of dung, in addition to digestive enzymes which may originally be present in the dung, bacterial enzymes are produced more rapidly in a dilute infusion as employed in the puer wheel, than in the dung itself. The enzymes are of various kinds, proteolytic, peptolytic, lipolytic etc., but the proteolytic and lipolytic are the most important. The have a solvent action on the fibres of the skin but little or no action on the hyaline layer at the concentration usually found in the puer liquor. The fatty matters and soaps in the skin are acted upon by the lipolytic enzymes and the fats are to some extent emulsified, so that they may be easily removed from the skin by scudding or pressing.

It must be clearly understood that enzyme action alone is not sufficient but that the dung enzymes acting

in conjunction with the chemical compounds present produce the specific effect.

The author has not tried to explain the action of of lipase as its action is only limited to the splitting of fat found in the animal tissue and which must necessarily be removed before a good tannage is obtained.

XXV

LAC SECRETION AND SYMBIOTIC FUNGI.

BY

S. Mahdihassan

AMONGST chemists who have analysed different plant products, there are two divergent opinions on the nature of their origin. According to one view, plants possess a rigid biological constitution and any qualitative difference among the chemical constituents must be ascribed to a difference in the botanical species or varieties. According to the other, they are endowed with a plastic constitution and the influence of climatic factors and soil are sufficient to account for a wider range of difference in the products synthesised by the same individual species. The chemical examination of different sorts of stick-lac also gives rise to two such antagonistic views. In this case, however, there are at least three factors, the species of lac-insect, the nature of the food plant and the temperature and humidity directly affecting the insect and again indirectly through the tree. However, there does not appear a thorough correlation between all the data collected by the chemist on the one hand and the entomologist or the botanist on the other, i. e. the insect or the tree does not always produce lac products specific to either or even to both when jointly considered.

When the lac insect is crushed and its body fluid observed under the microscope, just as blood-smears are examined in a public-health laboratory, yeast-shaped cells are seen. On culturing, these do not appear to belong to *Saccharomyces* or yeasts proper, but to different fungi. To a certain extent these symbiotic fungi seem characteristic of the genus of lac-insects and where

there is an exception the nature of the lac-exudation also shows wide qualitative difference. In a previous paper I have shown that the insects which produce stick-lac of commerce are truly Indian in their distribution. The genus including them should be known by the new and suggestive name of *Lakshadia*. The blood-smears of all species of *Lakshadia* show similar symbiotic fungi and are morphologically indistinguishable from one another. The genus *Ceroplastes* comprising different wax-insects also shows a parallel phenomenon. The symbiotic fungus associated with Indian lac-insects is most allied to *Monilia niger* isolated by the American Chemist Browne, from cuban cane sugar.

In Ceylon there are two lac-insects which support local industries of minor importance. Entomologically considered, one of them is the nearest ally of the Indian lac-insect and is named *Tacchardia albizziae*. The products of secretion also resemble the Indian stick-lac. The fungus of this Ceylon insect is most allied to *Oospora candida* which is a new name given by the American Mycologist Surastine for the famous fungus *Monilia candida*, known to impart fruity flavour to some wines. The change in nomenclature from *Monilia* to *Oospora* would show the resemblance the symbiotic fungus of *Tacchardia albizziae*, being an *Oospora*, bears towards that of *Lakshadia* species, which is a *Monilia*. In this case the difference between the morphology of the insects *Lakshadia* and *Tacchardia*, and the constituents secreted by them together bear a harmonic relationship with the difference found between the fungus of the Indian and Ceylonese lac-insects.

The other Ceylonese lac-insect, according to the latest nomenclature by Cameron is called *Meta-tacchardia conchiferata*. This insect is quite distinct from the others; its body colour is yellow, resembling

Ceroplastes insect; its secretion is almost glass-like and transparent. Its symbiotic fungus is a species of *Actinomyces* or ray-fungus as they are called and as such in its turm shows no relation with the genus *Oospora* or *Monilia*, already considered.

In Mysore we have two insects whose secretion does not melt in the same sense as shellac does. Their product has therefore never been utilised for any industrial purpose. I call them *Pseudo*-lac-insects and they now belong to the new genus Tacchardina of Cameron. One of them is *Tacchardina lobata*. Its secretion is purple and even bluish in colour while its symbiotic fungus, which looks like a dot shaped coccii, is really a fungus of the genus *Atelosaccharomyces*. This fungus never shows any formation of mycelium and as such is morphologically distinguished from the genus *Oospora*. It may be mentioned here that the symbiotic fungi of the ceroplastes insects are a species of *Atelosaccharomyces*. The fungus of *T. lobata* has, therefore, more resemblance to wax-insects than to other lac-insects and explains the great qualitative difference between stick-lac proper and *pseudo*-lac encrustation.

The other insect of this class is *Tacchardina Silvestrii*. Its exudation is amber coloured and as such can be easily distinguished from that of *T. Lobata*. The symbiotic fungus of *T. Silvestrii* looks like a rod shaped bacteria but on culturing proved to be a species of the fungus *Nocardia*. In the genus Tacchardina, we have two insects showing morphological similarity but great qualitative difference in their secretion products. This finds a rational explanation when we take into account the nature of their symbiotic fungi. *Tacchardina lobata* giving a purple secretion contains a species of *Atelosaccharomyces*. *T. Silvestrii* giving yellow secretion contains a species of *Nocardia*.

Although lac-insects must be classified according to their intrinsic morphological and physiological properties, yet their secretion products seem to bear a direct relationship with their symbiotic fungi. The study of this symbiosis has enabled a generic classification to be made between the allied lac-insects, *Lakshadia* and *Tacchardia* and between the species of the same genus *T. lobata* and *T. silvestrii*. It further lends itself to the view that the different secretory glands amongst these insects depend for their initial material upon the produce of these fungi, and the biogenesis of lac-products must start from the consideration of products elaborated by these fungi.

XXVI

SYMBIOSIS OF SEEDS AND BACTERIA.

Miss R. K. Christie, M. Sc.

INTRODUCTION.

SYMBIOSIS is the living together of two kinds of organisms to mutual advantage. Numerous examples of this phenomenon are encountered in both animal and vegetable kingdoms; a few of each kind may be mentioned here.

Symbiosis in animals:— Several investigators have found algae as symbionts in various animals, again insects have been found to live symbiotically on other insects. It has been proved that luminescence in animals and insects is due to parasitic organisms and symbionts.

Symbiosis in Plants:—The outstanding example of symbiosis in plants is the existence of lichens. These are compound organisms composed of a fungus and an alga. The combination is so perfect that it produces well characterised plants which were classified and studied separately, without the least suspicion of their dual nature, which, however, was discovered by Schwendener in 1867. In the formation of the consortium of the lichens. the algal cells become enveloped by the mycelium of the fungus. The fungus derives its nourishment saprophytically from the organic matter produced by the assimilating alga which in its turn derives a definite advantage from its consortium with the fungus, receiving from it inorganic subtances and water and possibly organic substances also. As a result of this existence, the healthiness, the longevity, the general usefulness and success of this compound, far exceeding anything fungus or alga could achieve singly, are remarkable.

Another well-known example of symbiosis in plants is afforded by the presence of bacteroids in the root-nodules of leguminous plants. These micro-organisms are capable of fixing atmospheric nitrogen and rendering it available for assimilation by plants. Nodules on the leaves of certain plants contain bacteria which are capable of fixing atmospheric nitrogen and hence such leaves become of prime importance as fertilisers.

Similar to bacteria, micorhiza also exist symbiotically with plants. The presence of the fungus mycelium in the roots of orchids was first noted in 1846 by Reisseck who also made an attempt to culture the fungus. It is to the researches of Noel Bernard (1902 onward), however, that we are actually indebted for a complete demonstration of the true relation existing between orchids and micorhiza, based as it is upon physiological studies. The important discovery of Bernard was that orchid seeds do not germinate in absence of fungi belonging to the genus *Rhizoctonia*. Similar explanation is given to the origin of tubers.

Thus symbiosis implies work and systematic service of organism by organism and from the above examples it is clear that the phenomenon of symbiosis of plants with bacteria and micorhiza present in root, leaf or seed, is a general one and that consequently it is of importance to the life of the plant.

The present paper deals with studies relating to symbiosis between seeds and bacteria. It has been found that paddy and cassia-tora seeds have specific bacteria associated with them, and that the presence of other bacteria is eliminated by means of an antiseptic characteristic of the seed. The seeds used in the present investigation are indigo and poppy, these being of economic importance. Indigo seeds used were of the *Sumatrana* variety, hard and brown, and consisting of three main parts, namely, the outer brown testa,

the middle semi-transparent endosperm which swells and gives a mucilaginous liquid on soaking in water and the yellow inner embryo.

Chemical examination of the seeds showed that the seeds contained tannins, sugars and a glucosidic substance. Starch was not found, the sugars obtained by hydrolysing the mucilage, were glucose and mannose, and as mannans form a constituent of the cell-walls of endosperms of various seeds, it is probable that the mucilage of the indigo seeds is a glucomannan. Furthermore, endosperms are reserve substances used by germinating seeds. This aspect was clearly observed in the present case, because the mucilage content of the seeds decreased with increase in the amount of Fehling reducing sugars formed as germination progressed.

The seeds on soaking in water were found to be fermenting, giving a mixture of carbon dioxide methane and hydrogen together with acetic and butyric acids.

In order to see if the fermentation was enzymic or bacterial, two lots of crushed seeds were set to ferment, after adding a few drops of thymol solution to one of them to prevent bacterial action but to maintain enzyme action. After 24 hours the contents of the flask containing no antiseptic fermented, while the other did not, showing that the fermentation was due to bacterial action. It was next thought necessary to find whether there were any specific bacteria which brought about this fermentation and what part of the seed was attacked by them. Bacterial investigation of the crushed and uncrushed sterilised seeds showed that only one type of organism, which under the microscope were seen to be short, motile rods and were spore formers, persisted, and were therefore considered to be specific to the seed. Cultures of these were then inoculated into the three main parts of the seed separately and also into mixtures of the three parts and it was found that the bacteria

were incapable of fermenting either of the three main portions of the seed separately or even the mixtures.

POPPY SEEDS.

The poppy heads, the seeds from which were under investigation, had not been lanced for the exudation of latex. When one of these poppy heads was cut open under aseptic conditions and a few seeds scattered on a nutrient agar plate, no bacterial growth was seen round the seeds except in the case of one or two, which were found to have a slightly ruputred skin. If, on the other hand, another portion of the seeds from the same capsule were crushed, observing asepsis and plated as above, abundant bacterial growth took place. It seems evident from this that the bacteria must have been enclosed by the seed. A very striking phenomenon observed in these experiments was that in almost all cases the seeds crushed without water gave very little bacterial growth, whereas the crushed material when supplied with water, gave abundant bacterial growth. This may be due to the dilution of some obscure antiseptic present in the seed.

Along with the above experiments a few seeds were scattered on a nutrient agar slope with a few drops of sterile water and set aside to see if germination of the seeds took place, and it was noticed invariably that wherever germination took place there was no bacterial growth round the seeding; but wherever germination did not occur either towards the top (drier portion) or towards the base of the slope (wet), bacterial growth was seen. Assuming that the bacteria are present in the seed to prepare food for the young seedling, or that they are used by the seedling as food—a possibility in harmony with the fact that every organism feeds on the ones lower than itself in the scale of evolution or that their function is to help germination by opening the testa of the seed by their fermentative activity or otherwise, or

that they are responsible for all the three functions mentioned, the absence of bacterial growth wherever good germination of the seeds has taken place might prove that when the bacteria remain inside the seed and do their duty whatever it may be, the seeds germinate, whereas when they fail to do it and leave the seed, these naturally fail to germinate.

The same type of organism was obtained from poppy seeds bought at the local market and also from poppy grown at the Institute garden. It was further observed that the bacteria are not present in the poppy seeds at very early stages of the bud, but reach the capsule by way of the stem, as the size of the fruit increases.

Cassia Tora, barley, annatto, linseed, castor and tomato seeds were next investigated for their internal bacterial content, with a view to finding if other seeds besides poppy held bacteria inside them. None of these were found to hold them, however, except barley; others had them residing on the surfaces.

Since bacteria have been found to be symbiotic with seeds in several cases, the next question arises as to what could be their function. A few examples of the existence to mutual advantage of two organisms constituting symbiosis have been given in the introduction to this paper. The cultural characteristics of the organisms under investigation did not give any clue as to the reason of their being symbiotic with seeds. The investigation was therefore extended in various directions in order to gain insight into the matter. The following inquiries were made:—(1) Whether the bacteria helped in the germination of the seed (2) Whether they promoted growth of seedlings; (3) Whether they fixed atmospheric nitrogen; (4) Whether they prepared food for the plant by breaking down the complex seed proteins into simpler substances assimilable by the plant.

Helping germination.—According to Nilson (*J. Am. Ch. Soc: 1904*) germination of barley is brought about by the agency of the lactic acid bacilli present in the seed and not by enzymes which are formed as a result of germination. His views, however, are contradicted by Windisch and Schonwold (*Woch. Bran.*, 1905. 22. 200) S. U. Pickering, (*Jour, Agr., Sc., Vol*: 11, 1907-8) has been successful in obtaining germination of different types of seeds on agar, under sterile conditions. In the present investigation, germination of indigo, cassia-tora, linseed, annatto, and tomato seeds was quite successful under perfectly sterile conditions. In one case, casia-tora seeds were allowed to germinate under sterile conditions, and after the seedlings had grown for about a week, sterilised nutrient agar was poured carefully down the sides of the tube in which the seedings were growing, so that if there were any bacteria connected with the germination they would have made their appearance on the nutrient medium. The seedlings continued to grow quite healthily, however, without there being even a trace of bacterial growth on the agar, showing that bacteria are not essential to the germination of the seeds.

Helping growth of Seedlings.—Though bacteria were found not to be essential to the germination of seeds (1) they seem to help their growth as shown by the experiment. When two groups of sterilised indigo seeds and one of unsterilised seeds, were set to germinate after watering one of the sterilised groups with sterile tap water, the other with washings of unsterilised seeds, and the unsterilised group with ordinary tap water, the difference in the growth of the seedlings was remarkable. Best growth was seen in the unsterilised group, next in order stood the sterilised seeds watered with washings of unsterilised seeds and poorest growth was observed in the sterilised seeds watered with sterile water.

3. *Nitrogen fixers.*—Indigo seed bacteria were found not to fix atmospheric nitrogen.

4. *Breaking seed proteins into simple substances.*—When indigo and poppy seed bacteria were inoculated into crushed seeds to which they were specific, they were found to break down the proteins into ammonia. It would seem therefore that they break the complex seed proteins and convert them into simpler substances assimilable by the seedling.

It has been observed before, that some seeds have bacteria specific to them and that the presence of other bacteria is eliminated by the presence of a selective antiseptic contained in the seed. The seeds under investigation, very likely, contained similar antiseptics. In order to see whether it was so, the effect of concentrated aqueous and alcoholic extracts of indigo, poppy and tomato seeds, poppy head, and rice polishings, were tried on *Bacillus coli*, tomato disease bacteria monilia and yeast. The method used was Delepine's silk thread method, slightly modified to suit our conditions of work. It was observed, contrary to expectation, that the extractives did not have a lethal action on the organisms, but had produced spore-formation in most of them. It seems therefore that seeds have a means of protecting themselves against the ravages of harmful bacteria by rendering them inactive, by means of the selective antiseptic contained in them.

It was further found that seeds contained some substance which induced growth of seedlings as well as of moulds.

In summarising, it seems, that seeds have bacteria symbiotic with them, and that their functions vary according to the requirements of the host.